DIRECTORATE OF FORENSIC SCIENCE SERVICES, MINISTRY OF HOME AFFAIRS, GOI ©

NEW DELHI

WORKING PROCEDURE MANUAL: CHEMISTRY

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FOREWORD

Forensic Sciences have a critical role to play in criminal justice delivery system. Successful prosecution of offenders hinges on the quality of test reports. Exhibits in crime cases need to be examined timely, precisely and accurately. In order to standardise and benchmark forensic analysis and test reports in respect of crime cases in Forensic Science Laboratories across the country, the Directorate of Forensic Science Services (DFSS), Ministry of Home Affairs has taken the initiative to prepare Working Procedure Manuals (WPMs) for various forensic disciplines.

Due to advancement of technology and its global access, crime is continuously evolving and adapting. New crime trends are emerging with people committing crimes in cyberspace, trafficking occurring in new psychoactive substances and drugs etc. Therefore, incorporation of new technologies in the WPMs to meet the ever increasing challenges for solving crime is the need of the hour, which is systematically and comprehensively reflected in these WPMs.

A long-felt need for such uniform WPMs has been fulfilled and I hope that these will be of immense use to forensic professionals of India. I congratulate the DFSS team for developing these manuals and urge them to keep on updating these at regular intervals.

Punya Salila

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PREFACE

The analytical procedures for examination of forensic physical clue materials in forensic science laboratories involve high degree of skill & expertise and play a significant role in a wide range of legal proceedings. The occurrence of error(s) in any of the forensic analytical activities is a serious matter for both laboratories and end users. For a laboratory, it can lead to re-testing of samples, if available, and loss of its credibility. The analytical techniques adopted by the scientist(s) for the forensic analysis may be one of the causes for this serious error.

The risk of committing error can be eliminated if the scientists undertake two or more independent validated techniques while conducting forensic analysis of crime case exhibits in the laboratory. Essentially, the procedures adopted must conform to the quality, sensitivity, repeatability and reproducibility of the examination so that the chances of error are absolutely avoided. It is, therefore, one of the essential requirements of good laboratory practices to introduce a Working Procedure Manual, which contains validated laboratory methods/techniques for forensic analysis of the exhibits. It is also necessary for all the Central/State Forensic Science Laboratories to follow these manuals in the country to maintain uniformity in test reports.

Keeping in view the advancement in science & technology and use of various protocols & procedures in the international arena of forensic science, the Directorate of Forensic Science Services (DFSS), Ministry of Home Affairs(MHA), has taken the initiative for preparing a systematic and comprehensive Working Procedure Manual for the discipline of 'Forensic Chemistry' to bring uniformity and standardization in the examination methods. In this regard, DFSS/MHA formed Scientific Working Groups, comprising eminent forensic scientists from the CFSLs and FSLs for each forensic discipline to compile forensic analytical techniques in the form of Working Procedure Manuals. Several meetings were conducted with detailed deliberations among the scientists at National level and finally the manual has been prepared /updated in the present form.

I am sure that this Working Procedure Manual, which pertains to the discipline of 'Forensic Chemistry' will help the forensic science laboratories to continue to follow standard and latest updated procedures in the examination of clue materials as well as to adopt quality control/ quality assurance in the forensic practices and also for obtaining accreditation from National Accreditation Board for Testing and Calibration of Laboratories (NABL).

I understand that there is always a scope of improvement and perfection can be achieved with collective efforts, therefore, stakeholders are welcome to offer their feedback and suggestion, if any, in this regard.

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SECTION – 1
INTRODUCTION

The Central Forensic Science Laboratories under the Directorate of Forensic Science Services are multi-disciplinary laboratories in which the chemistry division receives samples for only chemical analysis. The analysts adopt procedures as mentioned in the existing working procedure manual but where matrices of samples vary, new methods of processing are adopted after due validation. To bring uniformity in the analytical protocols among Central Forensic Science Laboratories for each parameter, the Directorate desired to review the existing Working Procedure Manual on Chemistry (WPM-Chem). The purpose of this manual/guidelines is to help the analyst in carrying out standard analytical protocols, and to bring uniformity in the working procedures for the examination of crime cases in the chemistry division of the laboratories. These guidelines of test methods recommended in this manual are based on certain scientific principles/facts/experiments and selected after consulting the various books and publications etc. The purpose of the chemical analysis is to examine the crime exhibits by chemical examination for its identification to provide scientific evidence to the Criminal Justice System. Though some of the test methods for the chemical examination for general routine cases have been recommended in the manual but depending upon the situation and need, other validated test methods/techniques available in the scientific literature can also be referred/ followed for the examination of exhibits.

2. This manual suggests approaches that may help the forensic analyst to select a technique appropriate to the sample being examined. Each method described here can be expected to produce reliable analytical information with respect to the samples to which they are applied.

SECTION - 2

COMMON HYDROCARBONS IN FIRE DEBRIS(ARSON CASE EXAMINATIONS)

INTRODUCTION

Arson means fire incident where a criminal intent is involved. The fire with criminal intention is done for getting economic/financial gain, on dowry death or on mob violence. The cause of fire may be either with the use of explosives material or fire accelerants. The investigating officer /team collects various types of exhibits involved in arson incidents and send to the laboratory for their examination. Flammable petroleum products such as petrol, kerosene, diesel etc. are commonly used as fire accelerants in arson cases. Sometimes, alcohol paints/thinner, industrial solvent and other inflammable materials are also used in the incidents of arson. The burnt exhibits or substances from the scene of crime are collected and sent to the laboratory for examination.

Forensic Science Laboratories are frequently called upon to examine partially burnt/charred clothing for the presence of traces of fire accelerants though kerosene, is the most common fire accelerant frequently used, due to its ready availability as domestic fuel. In order to establish the evidence of homicidal cases, the material collected from the scene of offence such as carpet, pillow, mattress, bed sheet etc. either partly burnt or unburnt, wiping of the floor, soil from the dwelling require special treatment for extraction of fire accelerant & their analysis.

Title: Examination/Testing of arson cases

Scope: All the exhibits collected from the scene of crime & forwarded by the investigating agencies to laboratories.

Purpose: Detection of common inflammable products like petrol, kerosene, diesel etc. or their residues in the exhibits of arson cases.

Methods:

Simultaneous detection of Petroleum Products and their residue by Gas Chromatography[4].

Extraction: Suspected portions of the samples from the exhibits, such as burnt clothes, paper, wooden material etc. are taken in a suitable sized beaker and extracted with appropriate amount of Di-ethyl Ether. Small exhibits may be extracted as such. The extract is filtered through Filter Paper No.1 in an evaporating dish and allowed to evaporate to concentrate at room temperature in fuming chamber. If direct extraction of the exhibit is not possible, the swab of the exhibit with cotton piece can be used for extraction. However, steam distillation is preferable as Di-ethyl ether extraction may show some extra peaks due to presence of oils, colours and other unwanted extractable things present in the exhibits. The continuation of burning depends upon many factors. In the process of burning, the low-boiling point hydrocarbons of the petroleum products burn first and high boiling point hydrocarbons may remain partially unburnt in the exhibits of arson. The petrol, kerosene, diesel and their residues can be identified and differentiated based on the pattern of the GC-Chromatogram vis-a-vis the standard chromatogram for each of these products. The residues of the above petroleum products in arson cases can be identified based on the relevant portion of chromatogram, matching with that of standard sample of petrol, kerosene and diesel analyzed along with the questioned exhibit.

Steam Distillation: Depending upon the nature (shape, size and state) of the exhibit, the appropriate portion of the burnt or partially burnt exhibits such as paper, hair, different types of clothes, wood etc. are made into small pieces and are steam distilled[1]. Aproximately 50 ml of distillate is collected. Extract the distillate with approximately 30 ml. of any of the organic solvents like benzene, diethyl ether, n-heptane, n-hexane etc with 2-3 extractions. Combine the extracts and concentrate by evaporating the extract at room temperature to a small volume (approx. 0.5-1 ml). The standard samples of petrol, kerosene and diesel (by adding about 0.5 ml of each in distillation flask) and blank sample are also prepared simultaneously in the same way.

(i) Gas Chromatography: The concentrated extract is again mixed with small amount Diethylether and analyzed on packed coloumn with SE-30 (5%) with following conditions:

Detector: Flame Ionization Detector (FID)

Carrier Gas: Nitrogen

Flow Rate: 30 ml/minute

Oven Temperature Programme:

Starts from 55^0C to 240^0C increased @ 8^0C per minute and isothermal for 10 minutes @ 240^0C

Detector Temperature: 250^0C

Injector Temperature: 250^0C

(ii) Gas Chromatography[2]

Appropriate volume of the concentrated extracts of the exhibit, standards and blank samples are injected into a gas chromatograph with the following conditions: -

Column: Pack SE – 30, Apiezon L or its equivalent column, which can be used for the separation of petroleum products etc.

Detector: Flame Ionization Detector

Carrier Gas: Nitrogen

Flow Rate: 30 ml/minute

Oven Temperature Programme:

i) For petrol – Starts at 70^0C (isothermal for 5 minutes); increases with 5^0/minute upto 120^0C. (70-120^0C)

ii) For kerosene – Starts from 90^0C and continues at 10^0C/minute upto 220^0C (90-220^0C); hold for 5.5 minutes at 220^0C

iii) For diesel - Starts from 90^0C and continues at 10^0C/minute upto 240^0C (90-240^0C); hold for 10.5 minutes at 240^0C

Detector Temperature:

i) For petrol – 170^0C

ii) For kerosene – 230^0C

iii) For diesel - 240^0C

Injector Temperature:

i) For petrol – 170^0C

ii) For kerosene – 230^0C

iii) For diesel - 240^0C

(iii) Gas Chromatography[3]

Operating Conditions

Column: 6' x 0.125" SS

Packing: OV 1, SE 30 or Apiezon L or its equivalent column, which can be used for the separation of petroleum products etc.

Detector: Flame Ionization Detector (FID)

Carrier Gas: Nitrogen

Flow Rate: 30 ml/minute

Oven Temperature: Programmed from 60^oC to 250^oC at 4^oC per min.

Detector Temeprature: 275^0C

Injector Temp: 250^oC

Capillary Gas Chromatography [3]

Operating Conditions

Column: 50 – 100m Glass Capillary x 0.010" ID

Packing: OV 101, or its equivalent column

Detector: Flame Ionization Detector (FID)

Carrier Gas: Nitrogen

Flow Rate: 1 ml/minute

Oven Temperature: Programmed from 60^oC to 250^oC at 2^oC per min.

Detector Temperature: 275^oC

Injector Temp: 250^oC

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SECTION - 3

OILS AND FATS

INTRODUCTION

Oils and fats are normally sent to the laboratory in the context of any criminal activity associated with them or in theft cases or in illegal possession cases (under Essential Commodities Act). Principally they consist of mono- & di-glycerides of the higher unsaturated fatty acids and free fatty acids in varying amounts, higher alcohols, resins and small quantities of colouring matter. The fatty acids entering into the composition of oils are numerous and hence oils and fats have a wide range of properties. The oils obtained from the seeds of related species frequently show a close resemblance and in some cases, however, oils from related species may be quite distinct. Oils and fats find many uses in industry. They are used in large quantities for edible purposes and in the manufacturing of soaps and cosmetics. Unsaturated oils are also of great importance in the paint and varnish industries. Many oils are used in medicines especially Ayurvedic and Unani medicine. Sometimes, oils like castor oils are also used for lubricating purposes. It is rather very easy to study the parameters of a known oil and to see whether they fall within the specifications laid down by Bureau of Indian Standards for a particular oil or not. But it is certainly very difficult job to identify an unknown oil by determining its analytical parameters as many of them like refractive index, density is either very close or parameters like acid value, colour etc. have a very wide range depending upon the grade. The only parameter, which has important significance, is Iodine value, which is a measure of unsaturation and is a valuable mean of identification. In forensic laboratories, the oil or fat has to be identified from various types of crime exhibits where the sample may not be in liquid form but a spillage on some material. Some times it has to be compared with standard sample of oil or fat for which the control /standard sample of oil and fat may also required for the comparison purpose. Therefore, depending upon the state of the oil/fat, the method has to be used.

Title: Oils and Fats.

Scope: Profiling of various oils and fats of vegetable origin in crime case exhibits.

Purpose: To analyze the various types of oils and fats in various crime exhibits received in the laboratory.

Methods:

SAMPLING FOR THE ANALYSIS:

Oils in liquid state:

Filter the oil sample through Whatmann filter paper no.1 to remove any unwanted particle.

Oils in solid and semi solid-state:

Melt the solid or semi solid samples of oils and fats in an oven heated to 10^0c above the melting point of the oil. Filter the melted oil through Whatmann filter paper no.1.

CHEMICAL TEST FOR IDENTIFICATION

3.4.2.1 Detection of Ground Nut oil: [1]

Take one ml of the sample of oil in a conical flask, add 5 ml of 1.5 N alcoholic potash and saponify completely on a boiling water bath using an air condenser to avoid loss of alcohol. It takes about 10 minutes. During the saponification process, swirl the flask many times, cool, add 0.1 ml of phenolphthalein indicator. Neutralize the solution exactly by adding dil. Acetic acid (One volume of glacial acetic acid + two volumes of distilled water) followed by an extra amount of 0.4 ml of acetic acid. Mix 50 ml of 70% alcohol, fix a thermometer into the flask with the help of a cork in such a way that the bulb of thermometer is dipped in the liquid but does not touch the bottom of the flask. Heat the flask on a water bath until the temperature reaches 50^0C to become clear solution. Keep the flask for cooling in air with frequent shaking until the temperature of the solution comes to 40^0C. Appearance of turbidity in between 39 to 40^0C indicates the presence pure groundnut oil. Cool the flask with constant shaking in a cooling bath at $15^0 \pm 1^0$ C in such a way that the temperature falls roughly at the rate of 2^0C per minute. Note the turbidity temperature, which is the temperature at which the first distinct turbidity appears. This turbidity temperature is once again confirmed by a little further cooling which results in the deposition of precipitate. Dissolve the precipitate by heating the contents at 50^0C on a water bath. Again, cool as above and make an experiment for duplicate determination of turbidity temperature. Duplicate shall agree with in $\pm 0.5^0$C.

Reagents:

1. Alcoholic Potash (1.5 N):

Dissolve 8.4 g of potassium hydroxide in 100 ml purified rectified spirit. The solution may be kept in dark colour bottle preferably.

2. Purified/ Rectified Spirit:

Reflux 1.2 litre of rectified spirit for half an hour in a distillation flask containing 10g of caustic potash and 6 g of granulated aluminium or aluminium foil pieces. Distil and collect one litre after discarding the first 50 ml, this is purified, rectified spirit used for reagents.

3. Phenolphthalein Indicator:

Take 0.5 g of phenolphthalein and dissolve in 50ml of purified rectified spirit and mix the solution with 50ml of distilled water.

Detection of Sesame oil: [1]

Take 5 ml of the oil sample or melted fat in a cylinder or test tube with a glass stopper add 5 ml of hydrochloric acid and 0.5ml of furfural solution (2% freshly distilled furfural in ethanol). Fit the glass stopper and shake vigorously for two minutes. Keep the mixture separate. The appearance of pink or red colour in the lower acid layer indicates the positive tests for the presence of sesame oil. Confirm it by adding 5 ml of water and shake again. If the colour remains, the sesame oil is present but if colour disappears, then sesame oil is absent.

Detection of Cotton seed oil:

Take about 5ml of the oil sample or melted fat in a test tube and add equal volume of sulphur solution (1% w/v solution of sulphur in carbon disulphide and add an equal volume of amyl alcohol). Shake thoroughly and heat it on a water bath (70^0 to 80^0C) for few minutes with occasional shaking. To boil off carbon disulphide and stop foaming, keep the test tube in an oil bath at 110-115^0C for about two and a half hours. Appearance of red colour at the end of the period indicates the positive test for the presence of cottonseed oil.

Detection of Palmolein in Ground Nut oil:

In this test, the cloud point of the oil is measured. Take 60-75 gms of samples and heat it to 130^0C before its testing. Add 45ml of the heated sample into an oil sample bottle. Keep the bottle in water bath. Cool the bottle in water bath with stirring using the thermometer to keep the temperature uniform. When the sample reaches a temperature 10^0C above the cloud point, being stirring steadily and rapidly in a circular motion so as to prevent super-cooling and solidification of fat crystals on the sides or bottom of the bottle. Do not remove the thermometer from the sample at this stage. The test bottle is maintained in such a position that the upper levels of the sample in the bottle and the water in the bath are about the same. Remove the bottle from the bath and read the temperature. The cloud point is that temperature at which that portion of the thermometer immersed in the oil is no longer visible when viewed horizontally through the bottle.

This test is useful for the detection of palmolein in groundnut oil. Presence of palmolein over 10% in groundnut oil readily gives cloud at a higher temperature than that of groundnut oil due to the presence of palmitic glycerides in higher amounts in palmolein/palm oil.

Detection of Linseed oil:

Take one ml of the oil sample in a boiling tube of about 100 ml capacity. Add 5ml chloroform and about one ml of bromine drop-wise till the mixture becomes deep red. Cool the test-tube in an ice water-bath. Add to it 1.5ml of rectified spirit drop wise with shaking the mixture until the precipitate which is first formed just dissolves and then add 10ml of diethyl ether. Mix the contents and keep the tube in the ice water-bath for half an hour. Appearance of precipitate indicates the positive test for the presence of linseed oil.

Note: This test is not applicable for the detection of linseed oil in presence of mohua oil.

Detection of Rice bran oil in other edible vegetable oil:

Take 20ml of oil sample in a 100ml separating funnel. Add to it equal volume of aqueous potassium hydroxide solution (30%). Shake the content gently and

constantly for 10 minutes. Allow the separating funnel to keep for about 45 minutes for the separation of alkali layer. Take out the alkali layer and neutralize it with dilute hydrochloric acid solution. Neutralization is confirmed with blue litmus paper. Extract this solution with diethyl ether (20ml x 3 times). Wash the extract with distilled water and dry on anhydrous sodium sulfate. Evaporate the solvent on hot water bath and dissolve the residue in chloroform and use it for Thin Layer Chromatography with following conditions.

Stationary phase: - Silica gel G, 0.25mm thickness.

Developing solvent: - benzene and acetic acid (100:1).

Development: – 15cm

Visualization: – Iodine fuming

Observation: - Rf value of Rice-bran oil – 0.70 to 0.75 (Use of control sample of Rice-bran oil is recommended).

This spot is absent in all the commonly available eatable oils in the market. The Rice-bran oil in other edible vegetable oils can be detected by this TLC method up to a minimum of 5% level.

A. Detection of Castor oil in edible oils by Thin Layer Chromatography:

Stationary phase: - Silica gel G, 0.25mm thickness.

Developing solvent: - Petroleum ether (40-60^{0}C), diethyl ether and acetic acid (60:40:2, v/v).

Development: – 10cm

Visualization: – Iodine vapours

Observations: - Rf value of castor oil – About 0.25

Sample preparation: – Chloroform

A prepared sample of an oil containing 1% added castor oil may be used as a control sample for identifying the spot. The spot shall be noted in tank since it fades on removing the plate. This method has a sensitivity of 1%.

This method is specific for castor oil but rancid or oxidized groundnut oil gives a streak.

B. Detection of castor oil and its differentiation from rancid oils: -

Take about 5ml of suspected rancid oil sample in a round bottom flask. Add to it about 2 gms activated charcoal. Mix the content thoroughly and heat it on a boiling water bath for about half an hour with constant shaking. The bleached oil is filtered on a filter paper. The filtered oil may now be passed through a mini column made up of neutral alumina –10 gms using 50ml hexane as eluent. This bleached and neutralized oil is used for TLC as given in the detection of castor oil besides as reference standard.

Detection of Argemone oil in edible oils: -

Method 1 – Ferric chloride test

Take about 5ml of filtered oil and dissolve in 5ml of toluene in a stoppered glass test tube. Add to it 5 ml of concentrated hydrochloric acid. Shake vigorously and allow the acid layer to separate. Separate the acid layer with the help of separating funnel to a test tube. Add 1 ml of Ferric chloride solution (10 gm Ferric chloride in 10ml concentrated hydrochloric acid and add 90 ml of water). If required, may be filtered through the side wall of the test tube. Mix the reagent and acid layer well. This can be achieved by gently rotating the test tube between the palms of the hand. Heat the test tube in boiling water bath for about 10 minutes. Formation of needle shaped (straight/curved) reddish brown crystals in clusters on cooling indicates the positive test for the presence of Argemone oil. The method is not sensitive enough to detect argemone oil in less than 0.1% level.

Method 2: -

Thin Layer Chromatography

Sample Preparation

Take about 5ml of suspected oil sample in about 5 ml of diethyl ether in a stoppered flask. Add 5 ml of conc. HCl and shake the mixture vigorously for 10 minutes. Heat the flask on a hot water bath having temperature 40^{o}C for about 10 minutes. Separate the acid layer using separating funnel in a 10ml beaker. Keep the beaker on a boiling water bath and evaporate to dryness. Dissolve the residue in 1 ml of chloroform and acetic acid mixture (9:1) and used for TLC with following conditions.

Stationary phase: - Silica gel G, 0.25mm thickness.

Developing solvents: –

1. Butanol, acetic acid, and water (7:2:1).
2. Hexane and acetone (6:4)
3. Heptane

Development: – 10cm

Visualization: – U.V. Light

Observation: - Bright yellow spot at about Rf value 0.8 in system 1, 0.4 to 0.5 in system 2. The plate shows spot of blue fluorescence if it is seen under UV Light after sprayed with 20% aqueous Solution of sodium hydroxide. The above observations indicate the positive test for the presence of argemone oil. The detection limit is up to 50 ppm.

Method 3:-

High Performance Liquid Chromatography[2]

DETECTION OF KARANJA OIL (*Pongamia Glabra*)

Sample Preparation: Take about 20 ml of suspected oil sample in a 100 ml separating funnel and 10 ml of conc. HCl. Shake the mixture gently and constantly for 15 minutes. Keep the funnel aside for about ½ hour to separate the acid layer. Take out the acid layer in a beaker and keep it on a boiling water bath for the evaporation to dryness. Dissolve the residue in about 0.5 ml of chloroform and use it to carry out the TLC with following conditions.

Stationary phase: - Silica gel G, 0.25mm thickness.

Developing solvent: –

Petroleum ether (40-60^{0}C): diethyl ether: acetic acid (6:4:1).

Development: – 20 minutes

Visualization: – Dry the plate at room temp. and watch under U.V. Light

Observation: -

1. Appearance of 3 bluish green spots at about Rf values0.34, 0.22, and 0.17 on the plate indicates the positive test for the presence of Karanja oil. The detection limit is up to 0.01 %.
2. Development of a yellow color in the acid layer after extraction also indicates the positive test for the presence of Karanja oil.

3. Palm argemone oil does not interfere.

Method 4: The TLC may also be carried out directly on chloroform solution of the oil instead of extracted solution but the test may not be sensitive.

Detection of Mineral oils in edible oils:

Method 1- Take 22 ml of the 0.5 N alcoholic KOH solution in a conical flask, add 1ml of the suspected oil sample. Boil it on a water bath using an air or water-cooled condenser till the mixture becomes clear and free from any oil drops on the side wall of the flask also. Take out the flask, transfer the contents to a wide mouthed warm test tube, add 25 ml of boiling water along the side of the wall with care. Shake the tube lightly from side to side while adding water. The appearance of turbidity indicates the positive test for the presence of mineral oil.

Method 2 – Thin Layer Chromatography

Stationary phase: - Silica gel G, 0.25mm thickness.

Developing solvent: –

Petroleum ether (40-60^{0}C or 60-80^{o}C).

Development: – About 4 minutes, / 6 cm

Visualization: – Dry the plate in air at room temperature, spray with fluorescein solution (2% soln. Of 2',7'-dichloro-fluoresein in 96 % ethanol and watch under U.V. Light

Observation: - Appearance of yellow fluorescent spots on the solvent front indicates the positive test for the presence of mineral oil. The vegetable oil gives a yellow streak about 2-3 cm long from the base.

Estimation of rancidity in edible oils:

Method 1- Kries Test:

Take about 5ml of suspected oil sample and shake vigorously with 5ml of 0.1% Phlorogucinol solution in diethyl ether and 5 ml of conc. HCl acid for 20 seconds. Appearance of pink color indicates the positive test for the presence of incipient rancidity.

Method 2 – UV Spectrophotometric method:

Take an accurately weighed amount of oil sample in a 25 ml volumetric flask so that the absorbance of its solution in iso-octane lies between 0.2 to 0.8 using a 1 cm quartz cell. Scan the sample by UV Spectrophotometer using iso-octane as blank/reference between 220 to 320 nm. Sample gives three wavelength λ max. at 230,268, and 278 nm. For quantitation select the wavelength λ max. of maximum absoption near or/ at 230,268, and 278 nm, and the absorbance (A) at these points.

Specific Absorbance E_1 1% cm (λ max.) = Absobance (A)/C x d

Where C = concentration of the sample solution in gm/100ml

d = length of the cell in cm

Method 3 – FT-IR Spectroscopic method

Major peaks **(cm^{-1})** of some of the oils are given below

Mustard oil: 3007, 2924, 2854, 1747,1465, 1418, 1378, 1238, 1163, 1119, 968, 722.

Vegetable Fat: 3005, 2922, 2854, 1747, 1465, 1418, 1378, 1236, 1163, 1119, 1099, 967, 722

Groundnut oil: 3007, 2954, 2926, 2854, 1747, 1465, 1418, 1378, 1237, 1163, 1119,1099, 966, 722

Coconut oil: 2955, 2924, 2854, 1747, 1466, 1418, 1378, 1228, 1161, 962, 722.

DETECTION OF ACID AND COLORS IN EDIBLE OILS:

Detection of Hydrocyanic acid in edible oils:

It is present sometimes as an impurity in mustard oil. Take 15 –30 ml of suspected oil sample in a 250 ml conical flask, add about 50 ml of water and mix well. Add to it 15 ml of 10% tartaric acid solution and mix. Put the stopper to the flask, with a velvet cork from which hangs a picric acid paper (Soak the filter paper in a saturated aq. solution of picric acid, draining the excess liquid and dry the paper in air) of about 75 mm long wetted with a drop of 5% sodium carbonate solution. The flask is kept on a hot water bath by the side of the steam vent but not directly on the steam for 30to 45 min. The red colour on picric paper indicates the presence of hydrocyanic acid. Ignore the pink or reddish hue, which may at times, appears at the periphery of the picric acid paper.

Detection of coal tar oil soluble colors in edible oils:

Take about 5 ml of suspected sample of oil in test tubes, add about 15 ml of petroleum ether to each tube followed by 5ml of Hcl of different concentrations (4:1, 3:1, 2:1, and 1:1 HCl and water) to different tubes. Change in color indicates the presence of coal tar oil soluble colors in the sample.

Isolation and detection of oil soluble colors:

Take about 5ml of suspected sample of oil stoppered conical flask, Add to it 25 ml of hexane followed by 10 gms of silica gel of column chromatography grade and 2 gms of anhydrous sodium sulphate. Stir the mixture and keep for 5 minutes. Decant the solvent. Add another 25 ml of hexane and stir well and decant the solvent. Repeat the process 3-4 times with 25 ml of hexane and draining out the solvent each time to remove almost all the oil leaving behind the silica gel in the flask. Elute the coloring matter absorbed by the silica gel in the flask by shaking with 20 ml of diethyl ether 2-3 times. Collect the diethyl ether extract in a beaker. Evaporate the solvent on a hot water bath to concentrate, which can used to carry out the TLC with following conditions.

Stationary phase: - Silica gel G, 0.25mm thickness.

Developing solvent: –

Benzene, hexane and acetic acid (6:4:1).

Development: – 12-15 cm

Visualization: – Dry the plate at room temp. and heat the plate at 100^0C in an oven for 1 hour.

Observations: - Natural colors like carotenes fades away leaving oil soluble coal tar colors.

DETERMINATION OF PHYSICAL PROPERTIES OF THE OILS:

Determination of moisture and volatile substances:

Carry out the determination of moisture and volatiles in duplicate.

Method-1 Weigh 5-10 gms of suspected sample of oil or fat in a tared metal dish of about 7cm dia and 3-4 cm deep with lid. Mix the sample thoroughly by stirring. Loose the lid and heat in an oven at 103$\pm$ 2 °C for 3 hours. Take out the dish from the oven and close its lid. Cool it in desiccator having phosphorous pentoxide and weigh.

Heat once again for 1 hour and weigh. Repeat the process to get concurrent readings with a difference of 2 mg

% age of moisture = Weight Loss/Weight of oil taken X 100

Method-2 Weigh about 10 gms but accurately of suspected sample of oil or fat in a tared glass beaker with glass rod, cool it in a desiccator having phosphorous pentoxide and weigh. Heat the beaker with sample on an electric hot plate with continuous stirring by glass rod. The complete removal of moisture is indicated by the cessation of the rising bubbles and by absence of foam, which can also be tested by keeping a clean and dry watch glass on the top of the beaker and observe the water vapours, if any. Cool the beaker to room temperature in the desiccator and weigh.

% age by Wt. of moisture and volatiles content = Wt. Loss/Wt.of sample taken X 100

3.4.4.2 Determination of Specific Gravity: - Determine the specific gravity of the oil by specific gravity / density bottle or a pyknometer method at ambient temperature by noting down the weight of specific gravity bottle with oil, with water and the empty bottle.

$$\textbf{Specific Gravity} = \frac{\text{Weight of bottle with oil - Weight empty bottle}}{\text{Weight bottle with water – Wt of empty bottle}}$$

CHEMICAL CHARACTERISTICS

Acid Value: It is the number of milligrams of potassium hydroxide required to neutralise the free fatty acid present in one gm of the oil or fat. The acid value is determined by directly titrating the material in an alcoholic medium with aqueous sodium or potassium hydroxide solution.

Reagents:

a) Ethyl alcohol (95%v/v) or rectified spirit neutral to phenolphthalein indicator.

b) Phenolphthalein Indicator - Dissolve one gm of phenolphthalein powder in 100 ml of ethyl alcohol.

c) Standard aqueous alkali Solution – Aqueous potassium hydroxide or sodium hydroxide solution of 0.1or 0.5 N.

Procedure: - Mix the oil or melted fat thoroughly before weighing. Weigh accurately a suitable quantity (2.0 to 2.5 gms) of the sample of oil or fat in a 250-ml conical flask and add to it 50 to 100 ml of freshly neutralised hot ethyl alcohol, add about 1 ml of phenolphthalein indicator solution. Boil the mixture for about 5 minutes and titrate while hot with standard aqueous alkali solution. Shake vigorously during titration. The weight of the oil or fat taken and the strength of alkali used for titration shall be such that the volume of alkali required for titration does not exceed 10 ml.

Calculation: -

Acid Value = 56.1 V N / W

Where, V = Volume of standard alkali hydroxide solution in ml,

N = Normality of standard alkali used, and

W = Weight of the sample of oil or fat taken in gm.

Saponification value: - It is the number of milligrams of potassium hydroxide required to saponify completely one gram of oil or fat. The material is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali solution consumed for saponification is determined by titrating excess alkali with standard hydrochloric acid.

Reagents: -

a) Alcoholic potassium hydroxide solution: - Dissolve 35 to 40 gm of potassium hydroxide in 20 ml of distilled water, and add sufficient aldehyde free rectified spirit to make up 1000 ml. Allow to stand overnight, decant the clear liquid and keep in a bottle closed tight with cork or rubber stopper.

b) Phenolphthalein Indicator solution: - Dissolve 1.0 gm of phenolphthalein in 100 ml of rectified spirit.

c) Standard hydrochloric acid: -approximately 0.5 N.

Procedure:

Melt the sample, if it is not already liquid, and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure the sample is completely dry, mix the sample thoroughly, and weigh by difference about 1.5 to 2.0 gm of the sample in a conical flask. Add 25 ml of the alcoholic potassium hydroxide solution with pipette and connect the reflux air condenser to the flask. Heat the flask on water bath or an electric hot plate for not more than one hour. Boil gently but steadily until

the sample is completely saponified as indicated by absence of any oily matter and appearance of clear solution. After the flask and condenser have cooled somewhat, wash down the inside of the condenser with about 10 ml of hot ethyl alcohol neutral to phenolphthalein. Add about 1 ml of phenolphthalein indicator solution and titrate with standard hydrochloric acid. Conduct a parallel blank determination at the same time.

Calculations: -

$$\textbf{Saponification Value} = \frac{56.1\,(B-S)\,N}{W}$$

Where,

B = Volume of standard hydrochloric acid required for the blank in ml,

S = Volume of standard hydrochloric acid required for sample in ml,

N = Normality of the standard hydrochloric acid,and

W = Weight of the sample of oil/fat taken in gms.

Iodine value: - Iodine value is the number of gram of iodine absorbed by 100 gm of oil or fat. Many methods for its determination are in use. Out of these Wijs is widely accepted and recommended method by the BIS. It gives the information about the amount of unsaturation or number of double bonds in a fat sample.

3.4.5.3. a) Determination of Iodine Value (Wijs)

General: -The material is treated in carbon tetrachloride medium with a known excess of iodine mono chloride solution in glacial acetic acid (Wijs solution). The excess of iodine mono chloride is treated with potassium iodide and liberated iodine estimated by titration with sodium thiosulphate solution.

Reagents:

i) Potassium Dichromate (AR)

(ii). Hydrochloric **acid -** Concentrated

(iii). Potassium iodide solution - Prepare a fresh solution by dissolving 10 gm potassium iodide free from potassium iodate, in 90 ml of water.

(iv). Starch solution - Triturate 5 gm of starch and 0.01gm of mercuric iodide with 30 ml cold water and slowly pour it with stirring into 1 litre of boiling water. Boil it for three minutes, allow it to cool and decant the supernatant clear liquid.

(v). Standard Sodium Thiosulphate solution (0.1N) – Dissolve approximately 24.8 gm of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in distilled water and make up to 1000 ml and standardise it as below.

Weigh accurately about 5.0 gm of previously dried to a constant weight at 105 $\pm$ 2°C potassium dichromate into a clean one litre volumetric flask. Dissolve in water, make up to mark, and shake thoroughly. Pipette out 25 ml of this solution into a clean 250 ml conical flask. Add 5 ml of conc. HCl and 15 ml of 10 % potassium iodide solution. Allow it to stand in the dark for 5 minutes and titrate the mixture with solution of sodium thiosulphate solution using starch solution as an internal indicator towards the end. The end point is taken when the blue colour changes to green.

Calculate the normality (N) of the sodium thiosulphate solution as bellow

$$\mathbf{N} = 25\ W / 49.03V$$

Where,

W = Weight of the potassium dichromate in gm,

V = Volume of sodium thiosulphate solution required for the titration in ml

vi) **Glacial acetic acid -** Free from alcohol.

vii) Carbon tetrachloride

viii) Iodine Monochloride (ICl)

ix) Wij's Iodine Monochloride Soution

Dissolve 10 ml of iodine monochloride in about 1800 ml of glacial acetic acid and shake vigorously. Pipette 5 ml of this solution, add 10 ml of potassium iodide solution and titrate with 0.1 N standard sodium thiosulphate solution using starch solution as an indicator. Adjust the volume of the solution till it is approx. 0.2 N.

Procedure:

Melt the sample if it is not already completely liquid, and filter through a filter paper to remove any impurities and the last traces of moisture. Use absolutely clean and dry glassware. Weigh accurately by difference an approx. quantity of the oil or fat between the limits indicated in col. 2 and 3 of Table-1 into a clean dry 250/500 ml iodine flask or well ground glass stoppered bottle to which 25 ml of carbon tetra chloride have been added and agitate to dissolve the contents. The weight of the sample shall be such that there is an excess of 50 to 60 percent of Wij's solution over that actually needed. Add 25 ml of the Wij's solution and replace the glass stopper

after wetting with potassium iodide solution, swirl for intimate mixing and allow the flask to stand in the dark for 30 minutes in the case of non-drying and semidrying oils and one hour in the case of drying oils. Carry out a blank test simultaneously under similar experimental conditions. After standing, add 15 ml of potassium iodide solution and 100 ml of water, rinsing in the stopper also, and titrate the liberated iodine with standard thiosulphate solution, swirling the contents of the bottle continuously to avoid any local excess until the colour of the solution is straw yellow. Add one millilitre of the starch solution and continue the titration until the blue colour formed disappears after thorough shaking with the stopper on.

Table 1 : Weight of oil or fat for determination of iodine value.

Iodine value **(1)**	**Minimum Weight of sample gm** **(2)**	**Maximum weight of sample gm** **(3)**
Less than 3	10	10
5	5.077	6.346
10	2.5384	3.173
50	0.5288	0.6612
100	0.2538	0.3173
150	0.1700	0.2125
200	0.1269	0.1586

Calculation:-

$$\textbf{Iodine Value} = \frac{12.69\,(B - S)\,N}{W}$$

Where,

B = Volume of standard sodium thiosulphate solution required for the blank in ml,

S = Volume of standard sodium thiosulphate solution required for the oil / fat sample in ml,

N = Normality of the standard sodium thiosulphate solution,

W = Weight of the oil / fat sample taken for the test in gm.

Table 2: Properties of some commonly encountered Vegetable Oils.(**5,6)**

Veg. Oil	**Sp. Gravity 30° / 30°C**	**Refractive Index at 40°C**	**Sap. Value**	**Iodine Value (Wij's)**
Groundnut oil	0.909-0.913	1.4620-1.4640	188 –195	87 - 98
Coconut oil	0.915-0.920	1.4480-14490	245-260	7.5-10
Sesame oil	0.915-0.919	1.4645-1.4665	185-193	105-115
Mustard oil	----------	1.4646-1.4666	169-177	98-110
Cottonseed oil	0.910-0.920	1.4630-1.4660	190-198	98-112
Soyabean oil	----------	1.4650-14710	189-195	125-140
Sunflower oil	----------	1.4640-14800	188-194	100-140
Mahua oil	0.862-0.875	1.4590-1.4610	187-196	58-70
Ricebran oil	0.910-0.920	1.4600-1.4700	180-195	90-105
Palm oil	----------	1.4491-1.4552	195-205	45-56
Palmolein	----------	1.4550-1.4610	195-205	54-62
Castor oil	0.954-0.960	1.4659-1.4730	177-183	82-89
Linseed oil	0.923-0.928	1.4720-1.4750	190-196	170-202

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SECTION - 4

GOLD EXAMINATION IN CHEATING CASES

INTRODUCTION

Gold is a precious and valuable metal to the human being. The human beings are fascinated for their ornaments. These ornaments are sometimes polished for maintenance. The criminals often polish the ornaments with polishing powder and by using aquaregia solution. The gold is not soluble in most of the acids; it is soluble in aqua-regia which is a mixture of one part of nitric acid and three parts of hydrochloric acid. Generally, criminals fool the housewives in the name of cleaning and polishing their ornament and dissolve the gold from their ornament in aqua-regia during the process of cleaning/ polishing. This aqua-regia solution and gold etc. seized from the scene of crime by the police are referred to forensic laboratory under IPC Section 420. Also, gold plated ornaments or even articles made of brass are sold to the unassuming persons as original gold ornament. These exhibits are to be examined in the laboratory for the analysis of gold and the purity as well. Colour test and modern sophisticated analytical techniques/methods, depending upon the nature of samples and examination, are used for the analysis of samples in the laboratory.

Title : Examination of Gold in cheating cases

Scope : Items received in the laboratory for detection of gold and purity.

Purpose : Analysis of gold in crime exhibits

Sampling:

The aqua-regia soln, about 250 ml from the crime scene should be sent in glass bottle with plastic lid or plastic bottle.

Material such as polishing powders, about 250 gm from the crime scene should be packed in polythene bag and sent to the laboratory.

The questionable ornament supposed to be original for proof of golden ornament.

***Methods*:**

Test for aqua-regia:

Test the aqua-regia soln for acidic nature, nitrate ions and chloride ions.

Test for Acidic Nature:

a) pH Paper Test: Moist the pH paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the pH. pH less than 7 indicates the presence of acid and more than 7 indicates the presence of alkali.

Alternate Method

a) Litmus Paper Test: Moist the blue litmus paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the colour change of the paper. Colour changing from blue to red indicates the presence of acid. If red litmus paper changes to blue, then it is indicates the presence of alkali.

Test for Chloride[1.2] :

a) Silver Nitrate Test [1,2]: Take the appropriate portion of the exhibit in a test tube and add few drops of 0.2 M silver nitrate solution. A white curdy precipitate is obtained which is soluble in excess of ammonium hydroxide solution but insoluble in water and dilute nitric acid. If no such phenomenon is observed, then repeat the test by adding 1 drop of nitric acid before addition of silver nitrate solution.

b) Test with Sulphuric Acid[3]: Take the appropriate portion of the exhibit in a test tube and add few drops of conc. Sulphuric acid if required warm it. Hydrogen chloride gas is evolved, which can be tested by blue litmus paper turns to red or by formation of white clouds of ammonium chloride when a glass rod moistened with ammonia solution is brought near the mouth of test tube.

Test for Nitric Acid

Test for Acidic Nature:

a) pH Paper Test: Moist the pH paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the pH. pH less than 7 indicates the presence of acid.

Alternate Method

b) Litmus Paper Test: Moist the blue litmus paper with distilled water and impregnate it with exhibit as such or it's distilled water extract and observe the colour change of the paper. Colour changing from blue to red indicates the presence of acid.

Test for Nitrate: Depending upon the nature of exhibits and availability of resources, any one of the following methods can be used for the detection of nitrate.

Take the appropriate portion of the exhibit in a beaker, add distilled water, shake well and filter it. The filtrate may be used for performing the tests.

a) Ring Test[3]: (i) Add about 3 ml of a freshly prepared saturated solution of ferrous sulphate to about 2 ml of the nitrate solution (filtrate of the exhibit) in a test tube and pour 3-5 ml conc. sulphuric acid slowly down the side of the test tube so that acid forms a layer beneath the mixture. A brown ring will form where the liquids meet which indicates the positive test for the presence of nitrate.

(ii) Add about 4 ml of conc. sulphuric acid slowly to about 2 ml of the nitrate solution (filtrate of the exhibit) in a test tube and mix the liquids thoroughly and cool the mixture in tap water. Now a saturate solution of ferrous sulphate in added slowly down the inner sidewall of the test tube to form a layer on the top of the liquid. Formation of a brown ring at the junction of two liquids indicates the presence of nitrate.[3]

b) Diphenylamine Reagent Test[3]: To a small amount of diphenylamine reagent (dissolve 0.5 gm. Of diphenylamine in 85 ml conc. sulphuric acid and dilute to 100 ml with water) in a test tube add a small portion of the filtrate carefully to the side of the test tube, a blue ring at the junction indicates the presence of nitrate.

c) Ferrous Sulphate Test[4] : To a drop of filtrate on a spot plate, add a crystal of ferrous sulphate (pin head), a drop of conc. sulphuric acid is allowed to run in at the side. In the presence of nitrate, a brown ring is formed around the ferrous sulphate crystal.

d) Brucine Test[4] : To a few drop of filtrate, a solution of brucine (0.02% in sulphuric acid, prepare immediately before use) is added on a spot plate, in the presence of nitrate a red colour is developed, on standing it changes to yellowish red.

Analysis of Gold Polishing Powder

Generally, the gold polishing powder is reddish in color and shows the presence of inorganic radicals such as copper, sodium, potassium, ammonium, iron, chloride, sulphate, nitrate etc. the analysis of gold polishing powder is carried out by the methods used for the analysis of inorganic qualitative analysis. The gold polishing powder may also be tested for the presence of gold.

Extract the polishing powder with water and the water extract is tested for cations and anions.

Tests for ammonium ions:

a) Nessler Reagent Test [4]:

Take one drop of test sample or extract, add one drop of conc. NaOH solution (5gms in 5 ml water)[3] on a watch glass or in a small test tube. Take out a micro drop from this and kept on a filter paper and add to it one drop of Nessler reagent. Appearance of a yellow or orange red stain or ring indicates the positive test for the presence of ammonium ions.

Preparation of Nessler reagent [3]:

Solution 1: Dissolve 10 gms of KI in 10 ml of water.

Solution 2: Dissolve 6 gms of mercury(II) chloride in 100 ml of water.

Solution 3: Dissolve 45 gms of NaOH in water and dilute to 80 ml.

Now add solution 2 to solution 1 drop wise until a slight permanent ppt. is formed then add solution 3, mix and dilute with water to 200 ml. Keep it for overnight and decant the clear solution. The solution may be used for one month.

b) Take an appropriate amount of the suspected sample, add to it few drop of NaOH solution in a test tube and heat it. Smell of ammonia is observed. This can be confirmed by bringing a glass rod dipped in HCl acid on the mouth of the test tube. White fumes are produced.[6]

Test for Chloride[1.2] :

a) Silver Nitrate Test: Take the appropriate portion of the exhibit in a beaker add distilled water, shake well and filter it. Take few ml. of the filtrate in a test tube and add 1 drop of nitric acid followed by few drops of 0.2 M silver nitrate solution. A white curdy precipitate is obtained which is soluble in excess of ammonium but insoluble in water and dilute nitric acid.

b) Test with Sulphuric Acid: As mentioned under section 5.5.1.2 b

Test for Sulphate[3.5] :

a) Barium chloride Test: Take the appropriate portion of the exhibit in a beaker add distilled water, shake well and filter it. Take few ml. of the filtrate in a test tube and add few drops of dilute hydrochloric acid followed by 0.25 M barium chloride solution.White precipitate, which is insoluble in water, indicates the presence of sulphate.

a)Rhodizonate Test[3]: Take a drop of barium chloride solution (0.25M – 61.1 g barium chloride dihydrate diluted in 1 litre of water) on a filter paper and add a drop of fresh solution of sodium rhodizonate (5%). Reddish brown colour spot appears. Now add a drop of acid or alkaline test solution. Disappearance of colour spot indicates the positive test for the presence of Sulphate.

Test for Sodium ions:

Color Test:

Uranyl Zinc Acetate Test [7]:

Take a portion of the exhibit solution and neutralize it with acetic acid. Add few drops of uranyl zinc acetate reagent, shake/ stir with glass rod. Formation of yellow precipitate or cloudiness indicates positive test for the presence of sodium.

Preparation of uranyl zinc acetate [8]:

Take 10 gms of uranyl acetate in 55ml of water, 30 gms of zinc acetate, and 9 ml of acetic acid. Heat to dissolve and dilute with water to make up to 100 ml. Allow to stand for 24 hours, and filter.

Alternate method for the preparation of uranyl zinc acetate reagent [9]:

Solution A:

Take 10 grams uranyl acetate in 6 gms of 30% acetic acid. If necessary warm it, dilute with distilled water to 50ml .

Solution B: 30 grams zinc acetate is stirred with 3gms 30% acetic acid and dilute it with distilled water to 50 ml. Mix the above two solutions A and B. Warm if required. Add a trace of sodium chloride, keep it for 24 hours and filter. Filtrate is used as above reagent.

Alternate Methods for Sodium

Flame test [10]:

Take appropriate portion of the exhibit as such or its water (distilled) extract evaporate to dryness, moisten with a few drops of conc. Hydrochloric acid to make past. Take a small portion of paste with the platinum wire and introduce into the non-luminous flame of a semi-micro burner. A persistence golden yellow flame indicates the presence of sodium.

Alternate Method of Flame Test[11]

Take a platinum or Nichrome wire and wet it with conc. Hydrochloric acid and heat it in the non-luminous flame of the burner until the yellow colour of the flame disappears. Dip the wire into test exhibit solution as such or it's distilled water extract (or powder if exhibit is solid) and heat it in the non-luminous flame of the burner. Observe the color of the flame as above. A persistent golden yellow flame indicates the presence of sodium.

Test for Potassium ions:

i) Dipicrylamine Reagent Test [4]:

Prepare a drop reaction paper by soaking the filter paper in sodium dipicrylaminate reagent (0.2 g dipicrylamine in 2 ml of 2N sodium carbonate and 15 ml water) and dry in a blast of heated air.

Place a drop of neutral test solution on the drop reaction paper, dry it in a cuurent of hot air. Keep the paper in 0.1N nitric acid. Formation of red fleck or ring at site of the spot indicates the positive test for the presence of Potassium.

ii) Flame Test: Potassium gives violet color when it is tested by flame test as described in the testing of sodium.

Test for Copper:

i) Test with ammonium hydroxide

Take the test sample solution (dissolve the sample in water or dil HCl or conc. HCl or dil/conc. HNO_3 or aquaregia to form original solution) and add to it ammonium hydroxide. Appearance of blue color indicates the positive test for the presence of Copper.

ii) Test with Alizarin blue [4]:

Take a drop of reagent (saturated solution of alizerin blue in pyridine) in a depression spot plate and add to it a drop of test solution. Run a parallel blank test with water also. Blue color appears in both the cases. Now add 1-2 drops of acetic anhydride, the color in the blank test converts into yellow but remaining of blue-violet precipitate in test sample indicates the positive test for the presence of Copper.

Alternate Method:

Take 1-2 drops of test solution in depression spot plate and evaporate it to dryness. Add a drop of the reagent and followed by a drop of glacial acetic acid. Appearance of blue-violet color indicates the positive test for the presence of Copper.

iii) Flame Test: Copper gives bluish green color when it is tested by flame test as described in the testing of sodium.

Test for Iron

i) Test with Potassium ferrocyanide:

Take few drops of test solution or original solution, add to it a drop of conc. Nitric acid and boil for few minutes. Add Potassium ferrocyanide solution. Appearance of blue color indicates the positive test for the presence of Iron.

ii) Test with thiocyanate [6]:

Take few drops of test solution or original solution, add to it a drop of conc. Nitric acid and boil for few minutes. Add ammonium or potassium thiocyanate solution. Appearance of red color indicates the positive test for the presence of Iron.

The above tests can be performed on filter paper or spot tile as stated below.

Put a drop of test solution on a filter paper or spot plate; add to it a drop of Potassium ferrocyanide solution. Appearance of blue color on paper or plate indicates the positivetest for the presence of Iron.

Put a drop of test solution on a spot plate; add to it a drop of potassium thiocyanate solution (1%). Appearance of red color indicates the positive test for the presence of Iron.

Test for Gold

i) *Test with Rhodamine B[4]* :

Few drops of the exhibit solution is taken in a micro test tube and add one-two drops of HCl and 1-2 drops of rhodamine B reagent solution and mix properly. The test tube is shaken with about 8-10 drops of benzene. Appearance of red-violet to pink colour in benzene layer indicates the presence of gold. After about 1-2 minutes it displays an orange fluorescence if it is observed under quartz lamp.

Reagent:

1) 0.01 g. rhodamine B dye stuff in 100 ml water

2) 0.2% aq. rhodamine B solution (given under detection of gold in alloys, coating etc.) the sensitive and specific test for gold with rhodamine B.

ii) *Test with Oxalic Acid*[5] : An appropriate portion of the exhibit is generally heated till the NO_2 fumes are removed. This solution is made alkaline by adding sodium hydroxide solution. Solid oxalic acid was added and generally heated. Brownish black precipitate indicates the presence of gold.

Quantitative estimation of gold in aqua-regia [14]:

Treat 20 ml of aqua-regia solution with dil HCl and heat the resulting solution on wire gauze. Make the solution nitrate free and test for nitrate ion by adding a drop on spot tile, followed by conc. H_2SO_4 solution and then a pinch of brucine powder. Nitrate ion gives orange colour. Continue the addition of dil HCl and heating with bunsen burner on wire gauze till the solution is free from nitrate ions. Reduce the nitrate free ion solution with 5% hydroquinone solution in water and warm on wire gauze. Filter the resulting solution and the gold in precipitated form is washed with warm water till it is chloride free, and then ignites in silica crucible in furnace at 850°C for about 2 hrs. and then weigh the gold. This method estimates about 0.1 gm% (w/v) of gold in aqua-regia.

Alternate methods:

i) *ICP-AES Method*[15] : This method/technique is used for the qualitative and quantitative analysis of gold in various types of crime exhibits. It involves the sample preparation followed by ICP-AES analysis. Appropriate amount of solid/powder exhibit was dissolved in aqua-regia (3 parts Hydrochloric acid and 1part of Nitric acid). Dilute it in appropriate volume with distilled water and analysed by ICP-AES. Run a parallel blank sample along with the crime exhibit.

ii) Ion Chromatography

iii) Atomic Absorption Spectrophotometry (AAS)

iv) Atomic Emission Spectrography

v) Fluroscence-XRD etc.

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SECTION - 5

SODA-LYE AND ACIDS

Title: Examination of corrosive chemicals like hydrochloric acid, sulphuric acid, and nitric and alkalies in crime exhibits.

Scope: Acids, alkalis and other crime case samples having presence of strong acids & alkalis.

Purpose : To detect the presence of hydrochloric acid, sulphuric acid, and nitric acid and alkalies in crime exhibits received from various investigating and law enforcement agencies

Methods: The following methods are to be used for the detection of the constituents of the hydrochloric acid, sulphuric acid and nitric acid in the crime exhibits.

Test for Hydrochloric Acid

***Test for Acidic Nature*:**

a) ***pH Paper Test* :** Moist the pH paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the pH. pH less than 7 indicates the presence of acid and more than 7 indicates the presence of alkali.

Alternate Method

b) ***Litmus Paper Test*:** Moist the blue litmus paper with distilled water and impregnate it with exhibit as such or it's distilled water extract and observe the colour change of the paper. Colour changing from blue to red indicates the presence of acid. If red litmus paper changes to blue, then it is indicates the presence of alkali.

***Test for Chloride[1]* :** Take the appropriate portion of the exhibit in a beaker, add distilled water, shake well and filter it. Take few ml. of the filtrate in a test tube and add 1 drop of nitric acid followed by few drops of 0.2 M silver nitrate solution. A white curdy precipitate is obtained which is soluble in excess of ammonium hydroxide solution.

Test for Sulphuric Acid

***Test for Acidic Nature*:**

a) ***pH Paper Test*:** Moist the pH paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the pH. PH less than 7 indicates the presence of acid.

Alternate Method

b) ***Litmus Paper Test*:** Moist the blue litmus paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the colour change of the paper. Colour changing from blue to red indicates the presence of acid.

***Test for Sulphate[1]* :** Take the appropriate portion of the exhibit in a beaker add distilled water, shake well and filter it. Take few ml. of the filtrate in a test tube and add few drops of concentrated hydrochloric acid followed by0.25 M barium chloride solution. White precipitate indicates the presence of sulphate.

Test for Nitric Acid

***Test for Acidic Nature* :**

a) ***pH Paper Test* :** Moist the pH paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the pH. PH less than 7 indicates the presence of acid.

Alternate Method

b) ***Litmus Paper Test* :** Moist the blue litmus paper with distilled water and impregnate it with exhibit as such or its distilled water extract and observe the colour change of the paper. Colour changing from blue to red indicates the presence of acid.

***Test for Nitrate* :** Depending upon the nature of exhibits and availability of resources, any one of the following methods can be used for the detection of nitrate. Take the appropriate portion of the exhibit in a beaker and add distilled water, shake well and filter it. The filtrate may be used for performing the tests.

***a) Ring Test[2]*:**

(i) Add about 3 ml of a freshly prepared saturated solution of ferrous sulphate to about 2 ml of the nitrate solution (filtrate of the exhibit) in a test tube and pour 3-5 ml conc. sulphuric acid slowly down the side of the test tube so that acid forms a layer

beneath the mixture. A brown ring will form where the liquids meet which indicates the positive test for the presence of nitrate.

(ii) Add about 4 ml of conc. sulphuric acid slowly to about 2 ml of the nitrate solution (filtrate of the exhibit) in a test tube and mix the liquids thoroughly and cool the mixture in tap water. Now a saturate solution of ferrous sulphate is added slowly down the inner sidewall of the test tube to form a layer on the top of the liquid. Formation of a brown ring at the junction of two liquids indicates the presence of nitrate.

***b) Diphenylamine Reagent Test[2]*:** To a small amount of diphenylamine reagent (dissolve 0.5 gm. Of diphenylamine in 85 ml conc. sulphuric acid and dilute to 100 ml with water) in a test tube add a small portion of the filtrate carefully to the side of the test tube, a blue ring at the junction indicates the presence of nitrate.

***c) Ferrous Sulphate Test[3]* :** To a drop of filtrate on a spot plate add a crystal of ferrous sulphate (pin head), a drop of conc. sulphuric acid is allowed to run in at the side. In the presence of nitrate, a brown ring is formed around the ferrous sulphate crystal.

***d) Brucine Test[3]* :** To a few drop of filtrate, a solution of brucine (0.02% in sulphuric acid, prepare immediately before use) is added on a spot plate, in the presence of nitrate a red colour is developed, on standing it changes to yellowish red.

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SECTION - 6

TRAP CASES EXAMINATION

INTRODUCTION

Chemical indicators change color between acidic and alkaline solution. Law Enforcement Agencies exploit this property of chemical indicators to lay trap for illegal gratification in the form of transfer of cash between two persons. Indicators like phenolphthalein or anthracene are very useful for their white color powdery nature. Now-a-days phenolphthalein indicator is being used in most of the trap cases. The currencies are laced with the phenolphthalein powder and when the cash is exchanged between hands, by Locard's principle, the indicator also get smudged in the hands of the alleged money receiver. When the hands or any other belonging of the receiver is washed with colorless sodium carbonate solution, the latter changes to pink colour. These washings are collected and sent to the forensic laboratories along with other relevant articles to establish the presence of phenolphthalein which can be considered as vital evidence in the court. The pink color of this solution persists for some days or months depending on the quantity of the phenolphthalein and strength of the alkali solution. It gradually fades and sometimes becomes colorless at the time of trial in the court. However, this phenomenon can be explained by the scientist on scientific basis that the color of the phenolphthalein fades due to its breaking down into 2(4 –hydroxy benzoyl) benzoic acid and phenol in alkali medium.

As mentioned earlier, anthracene powder is also rarely used for this purpose in trap cases as it does not pose such problem of color fading and has an advantage because of its fluorescence property. The hands, clothes etc of the suspect can be immediately examined under u. v. light, violet/blue fluorescence[1&2] can be clearly seen. This proves direct contact of the suspect with currency notes. Pure anthracene exhibits blue fluorescence but impure anthracene due to presence of tetracene, naphthacene etc exhibits yellow with green fluorescence.

Title : Detection of Phenolpthalein indicator or Anthracene.

Scope: The washings and other exhibits involved in the trap are sent to the laboratory for their examination for the detection of phenolphthalein and for the

presence of sodium and carbonate ions. Sometimes, anthracene and Calcium Hydroxide are also being asked to detect when they were used in trap cases.

Purpose: Detection of phenolphthalein, sodium ions, carbonate ions, calcium ions, anthracene etc. depending upon the type of trap carried out.

Methods:

. When phenolphthalein powder is used:

The hand washings, bag washings, cloth washings etc of the suspect is collected in dilute sodium carbonate-water solution or lime water along with other relevant articles from the scene of crime such as currency notes, clothes, bags etc sent to the laboratory for the chemical examination.

In case of untreated objects, ethyl alcoholic wash/ extract of the appropriate portion of the exhibits can be taken for the examination for the detection of the phenolphthalein. Alternatively, dilute solution of alkali (sodium carbonate) in water can also be used for washing/extracting the exhibits. These washing shall be used only for the detection of the phenolphthalein and not for the detection of the sodium and carbonate ions.

In case of alkali treated objects, wash the appropriate portion of the exhibits with water and used for the detection of the phenolphthalein, sodium and carbonateions etc.

When anthracene powder is used:

Articles from the scene of crime such as currency notes, clothes, bags etc along with traces of powder collected by carefully brushing the suspected area of contact of accused shall be sent to the laboratory for the examination.

In case of the object of anthracene, the appropriate portion of the object/ exhibit (after examination under u.v. light) can be washed with ethyl alcohol for the examination.

Details of the Methods of analysis:

The following methods can be used for the examination of the trap case for the detection of required constituents depending upon the case history/ nature of the examination of the case.

Test for Phenolphthalein:

Color Tests:

pH Test[2,3] :

Observe the pH of the solution exhibit with the pH paper. More than pH 9 (pH range 8.3-10) with pink /red color indicates the positive test for the presence of phenolphthalein.

Acid –Alkali Test[3, 4]:

Take an appropriate portion of the exhibit solution. Add few drops dilute hydrochloric acid. The pink color of the exhibit disappears. Now add few drops of dilute solution of sodium hydroxide in water, the pink color reappears. If required, this test can also be performed on residue obtained after evaporation of ethanol extract of the exhibit, but in this case first add alkali solution and then acid. Appearing and disappearing of pink color indicates the positive test for the presence of phenolphthalein.

Extraction[5,6] :

Take appropriate amount of the exhibit solution. Make the solution acidic with the addition of dilute hydrochloric acid drop wise with stirring till pH is about 4 to 5. (Alternatively, till acidic to Congo red). Extract with 20-25ml of solvent ether two times and evaporate to concentrate and used for other tests. The ether extract can also be evaporated to dryness and the residue can be dissolved in ethanol, which can be used for other tests.

***Thin Layer Chromatography*:**

Stationary phase : TLC plate coated with silica gel G or silica gel G F_{254}.

Mobile phase : Any one of the followings:-

1. Benzene, Dioxan and acetic acid (75:15:10)[6]

2. Chloroform and Acetone (80:20)[7]

3. Ethyl acetate, Methanol and Strong Ammonia (27 to 30%w/w)[7]
(8.5:1.0:0.5)

Visualization: 1. Spray with dilute sodium hydroxide solution
2.Acidified potassium permanganate solution[7]
(1% soln.of potassium permanganate in 0.25 M sulphuric acid)
3. U.V. light.

Instrumental techniques

Spectrophotometric examination[5]:

Take a portion of the exhibit solution, filtered and scan to note its λ_{max} absorbance value by spectrophotometer in appropriate dilution using a standard solution of phenolphthalein in aqueous alkali (sodium carbonate) for comparison. The pink color of phenolphthalein in aq. Sodium carbonate solution gives the lambda max in between around 550-555 nm**.** Aqueous solution of sodium carbonate is used as blank solution for the experiment.

Other instrumental techniques like HPLC, FTIR, GC-MS are also being used for the detection of Phenolphthalein.

Alternate Test for Phenolphthalein:

***Folin*-Ciocalteu's reagent test[5]:**

Take about 1 ml of alcoholic extract of the exhibit obtained as per required extraction procedure. Add 1 ml of folin –ciocalteu reagent followed by 2 ml of 20% sodium carbonate solution. Blue color indicates the positive test for the presence of phenolphthalein.

Test for Anthracene :

Color Test :

Observation under u.v. light – Violet/blue/green fluorescence.

Thin Layer Chromatography[8]: Sample preparation : In ethyl alcohol

Stationary phase : Silica gel G

Mobile phase : (Any two)

1. Heptane
2. Hexane
3. Carbon tetrachloride

Visualization : 1. U.V. light

2. Formaldehyde-Sulphuric acid reagent

(0.2 ml of 37% formaldehyde solution in 10ml of conc. Sulphuric acid)

ALTERNATE METHODS

6.5.2.3 Separation and purification of Anthracene from seized material[9]:

Currency notes, shirts, pant, handkerchiefs, diaries, books etc in anti-corruption cases/bribe trap cases collected over a year were examined a long with commercial anthracene by TLC technique using Chloroform developing solvent. The suspected portion after locating under UV lamp of every exhibit was initially extracted in ethanol/ether, then subjected to TLC/GLC examination.

THIN LAYER CHROMATOGRAPHY[9]:

Sample preparation : In ethyl alcohol [95% v/v]

Stationary phase : Silica gel G [Activated at 110^0 C for 1 hour]

Mobile phase : Chloroform

Visualization : U.V. light

U V *Spectrophotometry[9]:*

The separated spot of the analyte corresponding to reference spot of anthracene was scrapped off from the preparative TLC. To the scrapped silica gel appropriate amount of ethanol was added. Filter or decant it. Concentrate the filtrate to appropriate volume. Blank was similarly prepared from the silica gel scrapped off from the same plate. The filtrate was subjected to UV spectroscopic study. Alternatively the exhibit can also be extracted with 95% of ethanol and subjected to UV Spectroscopy study. A control sample of anthracene in ethanol can be used as a standard sample for comparison of UV Spectrum.

Gas Liquid Chromatography[9]:

Detector : Flame ionization

Column : S S column 1/8 inch dia. 2 meter length

Packing : 10% S E 30, 80/100 chromosorb W-HP

Carrier Gas: Nitrogen

Flow rate : 25 ml/min

Injector temperature : 250^0C

Column temperature : 220^0C Isothermal

Detector temperature : 250^0C

Sample preparation: In ethanol

***Test for Carbonate ions* :**

***Color Tests* :**

***Test with acid*[10] :**

To a portion of the exhibit solution add few drops of dilute hydrochloric acid, effervescence is observed. If needed, the resulting gas can be passed through baryata water/ lime water. Turbidity/ curdy white precipitate appears. If the gas is passed for long time, the precipitate or turbidity slowly disappears. The exhibit may be gently heated with dilute hydrochloric acid to produce sufficient effervescence/ gas.

***Barium Chloride Test* [10]:**

To a portion of the exhibit solution add few drops of barium chloride solution (about 5-6 % barium chloride in water. Formation of white precipitate, which is soluble in mineral acids, indicates the positive test for the presence of carbonate ions. Bicarbonate ions do not form white precipitate, as they do not react with barium chloride solution.

***ALTERNATE METHODS*:**

***Silver Nitrate Test*[10]:**

To a portion of the exhibit solution add few drops of silver nitrate solution (about 2 % in water). Formation of white precipitate, which is soluble in ammonia solution, indicates the positive test for the presence of carbonate ions.

***Magnesium Sulphate Test*[10]:**

Take appropriate portion of the exhibit as such or its water (distilled) extract. Add magnesium sulphate in the cold condition. If no precipitate is obtained then it indicates the presence of bicarbonate. If a white precipitate is formed then it is a positive test for the presence of carbonate.

***Test for Sodium*:**

***Color Test*:**

***Uranyl Zinc Acetate Test*[11]:**

Take a portion of exhibit solution and make it neutral with acetic acid. Add few drops of uranyl zinc acetate reagent, shake/ stir with glass rod. Formation of yellow precipitate or cloudiness indicates positive test for the presence of sodium.

***Preparation of uranyl zinc acetate[12]*:**

Take 10 gms of uranyl acetate in 55ml of water, 30 gms of zinc acetate, and 9 ml of acetic acid. Heat to dissolve and dilute with water to make up to 100 ml. Allow to stand for 24 hours, and filter.

***Alternate method for the preparation of uranyl zinc acetate reagent* [13]:**

Solution A:

Take 10 grams uranyl acetate in 6 gms of 30% acetic acid. If necessary warm it, dilute with distilled water to 50ml .

Solution B: 30 grams zinc acetate is stirred with 3gms 30% acetic acid and dilute it with distilled water to 50 ml. Mix the above two solutions A and B. Warm if required. Add a trace of sodium chloride, keep it for 24 hours and filtered. Filtrate is used as above reagent.

Test for Calcium

Test with Sodium Rhodizonate[14]:

Take one drop of neutral or weakly acid test solution add a drop of freshly prepared 0.2% a sodium rhodizonate solution add one drop of 0.5 N sodium hydroxide solution, a violet colour indicates the presence of calcium.

Alternate Methods for Sodium & Calcium:

***Flame test[15]*:**

Take appropriate portion of the exhibit as such or its water (distilled) extract evaporate to dryness, moisten with a few drops of conc. Hydrochloric acid to make past. Take a small portion of paste with the platinum wire and introduce into the non-luminous flame of a semi-micro burner. A persistence golden yellow flame indicates the presence of sodium and a brick red (yellowish red) flame indicates the presence of calcium.

Alternate Method of Flame Test[16]

Take a platinum or nichrome wire and wet it with conc. Hydrochloric acid and heat it in the non-luminous flame of the burner until the yellow colour of the flame disappears. Dip the wire into test exhibit solution as such or its distilled water extract (or powder if exhibit is solid) and heat it in the non-luminous flame of the burner. Observe the colour of the flame as above.

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SECTION- 7

EXAMINATION OF LIQUOR/ ALCOHOLIC DRINKS AND ALCOHOL

INTRODUCTION

Liquor is normally a mixture of water and alcohol. The term alcohol is often used for ethyl alcohol. The liquor is manufactured by the fermentation process in which carbohydrates are fermented in presence of enzymes as per their specifications given in Bureau of Indian Standards (BIS). Country made liquor is alcoholic product usually prepared from fermentation of carbohydrates present in cereals, jaggery, fruits, mahua, palm, molasses etc. The liquors are sold in the market in various brands and covered under Excise Act. The possession, sale, transportation of liquor is allowed only as per the Rules and Regulations of Excise and Prohibition. On many occasions, these liquors are being smuggled from one State to another State, illegal possession, transported without proper valid documents. These samples are seized by the Police and submitted to the Forensic Laboratory for their examination. The liquor is examined in the laboratory for two purposes; firstly, for Excise purpose where, mainly the presence of alcohol plays an important role and accordingly the examination of liquor samples for the qualitative and quantitative analysis is the main purpose of the investigation. Secondly, the liquor is examined for quality control/duplicate samples, which are being sold in the market in which the examination is carried out for other parameters also apart from alcohol contents. The alcohol contents are also reported in percentage of proof spirit, percentage of alcohol (weight by volume) and percentage of alcohol (volume by volume).

Title **:** Testing of Alcoholic drinks/liquor/alcohol

Scope**:** Various types of alcoholic drinks/liquor in crime exhibits for excise and checking of quality control/duplicity purposes.

Purpose : To analyze the various types of alcoholic drinks to detect the presence and percentage of alcohol (ethyl alcohol)/percentage of proof spirit, and other ingredients in liquor, if required, in the crime exhibits received from various investigating and law enforcement agencies under Excise and Prohibition Act. This

generally prescribes the methods for test of alcoholic drinks/alcohol in crime exhibits, which is generally found in cases of illicit possession and preparation, smuggling, spurious/duplicate liquor, excise and other crime cases etc.

Methods:

***Qualitative Analysis of Liquor*:**

Test for Ethyl Alcohol: The following tests are to be carried out for the detection of ethyl alcohol in the exhibits.

(a) ***Iodoform Test***[1] : Take about 1 ml or appropriate sample (distilled or as such depending upon the nature of sample and concentration of ethanol) and add about 1 ml of 5% sodium hydroxide solution and then add iodine solution (20 gm Potassium iodide + 10 gm Iodine in 100 ml water) drop-wise with shaking until the liquid becomes persistent dark brown in colour. Keep it for 2-3 minutes. If the iodine colour disappears add more drops of iodine solution until persistent brown colour of iodine. Add few drops of dilute sodium hydroxide solution to remove extra iodine. Add equal volume of water, keep it for ten minutes. Yellow crystalline precipitate indicates the positive test for the presence of ethanol.

(b) ***Dichromate Test***[2] **:** To about 1 ml or appropriate amount of sample (distilled or as such depending upon the nature of samples and concentration of ethanol) is added about 0.2 ml of 2% potassium dichromate solution followed by about 1 ml of concentrated sulphuric acid. The yellow colour of the dichromate changes to green or blue indicates the presence of ethanol.

Test for Methanol[3] **:** Take about 1 ml or appropriate amount of sample (distilled or as such depending upon the nature of sample and concentration of methanol) in a test tube add about 2 ml of potassium permanganate solution (3 gm potassium permanganate and 15 ml of phosphoric/ortho phosphoric acid in 100 ml distilled water) and shake well. Now add few crystals of sodium bisulphate with shaking till disappearance of colour (potassium permanganate colour) of the solution. Add about 1 ml of chromotropic acid (5% of aqueous solution of sodium salt of chromotropic acid) and add concentrated sulphuric acid slowly with inner sidewall of the test tube to the extent of 15 ml. Appearance of violet colour indicates the presence of methanol.

Test for Copper & Iron[4] **:** Take about 5 ml or appropriate amount of sample add 1 drop of Nitric acid and 1 ml of 0.025 M potassium Ferrocyanide solution. Prussian blue colour indicates presence of iron and chocolate colour indicates the presence of copper.

Test for Furfural[3] **:** Take about 5 ml or appropriate amount of sample (distilled or as such depending upon the nature of sample and concentration of furfural) in a test tube, add about 1 ml aniline and about 0.5 ml hydrochloric acid and keep it for 15 minutes. Appearance of red colour indicates the presence of furfural.

Alternative method for testing furfural :

a) Take about 2 ml or appropriate amount of the sample distilled or as such depending upon the nature of sample in a test tube, add about 0.2 ml of aniline and about 0.4 ml of glacial acetic acid. If the furfural is present in the sample, red colour develops in a few seconds & reaches its maximum intensity in 5-10 minutes[5].

b) Take about 2 ml or appropriate amount of the sample distilled or as such depending upon the nature of sample in a test tube, add about 1 ml of aniline acetate solution (10% V/V solution of aniline in glacial acetic acid). Appearance of red colour indicates the presence of furfural. The colour develops at room temperature of 25-30^{0}C and reaches its maximum intensity in 1-5 minutes[6].

Quantitative Analysis of Liquor**:**

Determination of Ethanol**:** Depending upon the nature of sample and examination required and resources available any one of the following methods can be used for the quantitative analysis of ethyl alcohol.

Determine the specific gravity of the distilled sample by specific gravity bottle and apply the bottle correction and temperature correction for 60^{0}F as per the table showing weight in gram of the spirit of temperatures 61^0 to 100^0 given under the instructions for ascertaining the real alcoholic strength of drugs chemicals, medicines dietetics and toilet preparations entered for test prepared by R.L. Jenks, F.I.C., Examiner for Customs and Excise, Calcutta (Central Board of Revenue) 1914-1928 Revised-1936 and find out the quantity of alcohol (% of proof spirit and % of alcohol V/V) from the table showing the relation between the specific gravity of spirits at 60^0/60^0 F and the percentage of ethyl alcohol by weight and by volume with the

corresponding percentage of proof spirit issued under the authority of the Commissioner of Her Majesty's Customs and Excise, London, Her Majesty's stationery office 1955, Reprinted 1971 **(Annexure I & II)**.

Example of Calculation: At temperature of 76^0F (24.5^oC) F=CX1.8+32=**76.1ºF**

1. Weight of 50 ml specific gravity bottle : 26.6870 gm
2. Weight of bottle plus distilled water : 76.6720 gm
3. Weight of water : 76.6720-26.6870 = 49.9850 gm
4. Weight of 1000 ml water : 49.9850 x 20 = 999.7 gm
5. Temperature correction : 1.64 (to be added) at 76^oF
6. Weight of 1 liter water : 999.7 + 1.64 = 1001.34
7. Bottle correction : 1001.34 – 1000 = 1.34
8. Weight of exhibit and bottle : 73.8250 gm
9. Weight of the exhibit : 73.8250 – 26.6870 = 47.138 gm
10. Weight of the 1000 ml exhibit : 47.138 x 20 = 942.76 gm
11. Weight of exhibits with bottle correction : 942.76 – 1.34 = 941.42 gm
12. Temperature correction : 6.00 (at 76^0F)
13. Corrected weight of the exhibit : 940.46 + 6.00= 946.46 gm
14. Specific gravity of the exhibit : 946.46/1000 = 0.94646
15. Percentage of proof spirit : 75.69
16. Percentage of alcohol V/V : 43.25

ALTERANATIVE METHODS FOR ETHYL ALCOHOL:

(a) *Gas Chromatography*[1] :

Operating conditions :-

Column : Porapak polymer bead 80-100 mesh or its equivalent, which can separate/resolve the ethanol

Column Temperature : 160^0C

Carrier Gas : Nitrogen Gas

Rate of gas flow : 50 ml/min

Detector : FID (Flame Ionization Detector)

Alternative Operating Conditions[7] :

Column : 0.3% Carbowax 20 M on 80-100 mesh Carbopak C, 2m x 2mm ID or its equivalent

Column Temperature : 35^0C for 2 minutes and then programmed at 5^0C per minute to 175^0C and hold for at least 8 minutes

Carrier Gas : Nitrogen at 30 ml/min

(b) The percentage (contents) of the alcohol can also be found from the table given in Official Methods of Analysis of the Association of Official Agricultural Chemists (A.O.A.C.)[8] after calculating the specific gravity of the liquor samples.

Determination of Methyl Alcohol **:**

Gas Chromatography[1] **:**

Operating conditions :-

Column : Porapack polymer bead 80-100 mesh or its equivalent, which can separate/resolve the ethanol

Column Temperature : 160^0C

Carrier Gas : Nitrogen Gas

Rate of gas flow : 50 ml/min

Detector : FID (Flame Ionization Detector)

Alternative Operating Conditions[7] :

Column : 0.3% Carbowax 20 M on 80-100 mesh Carbopak C or its equivalent

Column Temperature : 35^0 for 2 minutes and then programmed at 5^0 per minute to 175^0 and hold for at least 8 minutes

Carrier Gas : Nitrogen at 30 ml/min

7.4.2.3 ***Other Determinations*****:** The following determinations (some or all depending upon the nature of examination and infrastructure available), if required, may be carried out as per IS:3752:1988.

i) Determination of ash

ii) Determination of total acidity

iii) Determination of volatile acidity

iv) Determination of fixed acidity

v) Determination of residues on evaporation

vi) Determination of esters

vii) Determination of higher alcohols

viii) Determination of aldehydes

ix) Determination of copper

x) Determination of furfural

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ANNEXURE- I

SOME OF THE IMPORTANT AND USEFUL REFERENCES OF BOOKS/LITERATURE:

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ANNEXURE- II

IMPORTANT TECHNIQUES:

1. Colour tests
2. Thin Layer Chromatography (TLC)
3. High Performance Thin Layer Chromatography (HPTLC)
4. High Performance Liquid Chromatography (HPLC)
5. Gas Liquid Chromatography (GLC)
6. Gas Chromatography – Mass Spectrometry (GC-MS)
7. Liquid Chromatography – Mass Spectrometry (LC-MS)
8. Emission Spectrography
9. ICP-OES
10. AAS
11. X-Ray Diffractometer (XRD)
12. Ion-Mobility Spectroscopy
13. Microscopy
14. UV-Vis Spectrophotometry
15. IR/FTIR Spectroscopy, etc.

SOME OF THE IMPORTANT AND USEFUL TECHNIQUES / INSTRUMENTAL METHODS:

1. High performance thin layer chromatography (HPTLC):

This technique is advancement of TLC, which is also called Modern TLC or Instrumental thin layer chromatography or planar chromatography. In this technique the sample solution is spotted on TLC/HPTLC plate with the help of automatic sample applicator in band or spot form. The band wise application gives better separation. The plate is developed in suitable developing system depending upon the nature of the sample in appropriate developing chamber. The most commonly used chamber is twin trough chamber. After the development of chromatogram, the plate is scanned by scanner for getting densitogram followed by spectral scanning, if required, in uv-vis regions. After the densitometric evaluation of the chromatogram

and spectral scanning, the identity of the compound in test exhibit or mixture can be confirmed based on Rf value, pattern and λ-max of the in-situ spectrum is matched with control or standard sample. The quantitative analysis can also be carried out based upon the peak area or peak height calculation. The technique can also be used to know the purity (peak purity) of the compound qualitatively.

2. High performance liquid chromatography:

Principle-In this technique mixture of sample is separated on columns, filled with small particles. (10 μm in diameter) by elution with liquid under high pressure. The two important types of chromatography are (1).Adsorption chromatography
(2).Partition chromatography.

In adsorption chromatography, column is packed with solid silica, Alumina that retains the solute by adsorption. It is also called liquid solid chromatography. In partition chromatography column is packed with thick layer of liquid & sample get partitioned between two liquids, it is also called liquid-liquid chromatography. When sample is injected to the column separation of sample to components takes place with the help of mobile phase (a liquid phase). These components pass through detector generally refractive index or ultra violet, which detects specific property of component which is not possessed by mobile phase, signal produced are fed to the integrator which records chromatogram. Preparation of sample is made by dissolving solute in suitable solvent & injected to column, which is previously rinsed with mobile phase. The sample gets partitioned between mobile phase & stationary phase or get adsorbs on stationary phase, depending on type of chromatography and separates into component. The separated component passes through detector where signal is produced, signal is fed to integrator, that record chromatogram. H.P.L.C. technique is generally used for substance, which are thermally labile such as explosives, cannabinoids as it works at ambient temperature. It is also used for polar drugs requiring derivatisation on GLC systems can be assayed directly on H.P.L.C.

3. Gas chromatography:

Gas chromatography, like other chromatography is method of separating mixture of sample of analytical interest. Separation is performed on column containing

stationary phase either solid or liquid, which is maintained at definite temp. in an oven & has constant flow of carrier gas, a mobile phase. When mixture of sample is injected at inlet each component is swept towards the detector & gets partitioned between gas phase & stationary phase. The molecules having greatest affinity for stationary phase spend more time in that phase & hence take longer time to reach the detector. Detector produces a signal dependent on the mass of component passing through it. This signal is processed and fed to a chart recorder and then to the integrator. Each component passing through column will have characteristic retention time at given conditions. There are two types of column generally used in gas chromatography. (1) Capillary column. (2) Packed column. Capillary column has superior resolution over packed column, but the choice of phases for capillary is limited over packed column. In forensic context gas chromatography is applicable for many compounds such as drugs, unknown chemicals, petroleum products, gases like H_2S, N_2O & anaesthetics. Preparation of sample is made by dissolving solute into suitable solvent & then injected to preheated column with micro syringe through silicon rubber septum. Clean extract should be used to minimise contamination of column. With the help of gas (a mobile phase) the components of sample are swept to detector. Chromatogram of sample along with standard markers helps in identification & quantitation of the compound.

4. Gas chromatography mass spectrometry (GC / MS):

In this technique gas chromatography is coupled with mass spectrometer GC separates the mixture into component, based on retention of analyte between two phases (liquid stationary phase and mobile gas phase). The interface directs the GC effluent into mass spectral detector. Where electron ionisation and subsequent fragmentation of molecule take place. The ions of negative & positive charges are produced, the mass filter quadruple sorts these ions based on mass/charge (m/z) ratio. The functional group present in the molecule directs fragmentation in such way that structure of original molecule can be predicted by fragmentation pattern. The detector produces the signal, which is proportional to number of ions of particular m/z ratio produced. Signal feeds to recorder that record chromatogram. Preparation of sample is done by dissolving sample into suitable solvent. Clean extract is injected to column

with microsyringe, splitless injections are most common in GC/MS analysis. With the help of mobile phase (gas) component of sample are swept towards interface. From interface component are directed to mass spectral detector (M.S.D.), where ions are produced & signal obtained fed to the recorder, which records chromatogram for separate component. Main advantage over gas chromatography is that GC/MS helps in determination of molecular weight of component which are separated.

5. UV-VIS Spectrophotometry:

When monochromatic light beam passes through a transparent medium the rate of decrease in intensity with increase of thickness of medium is proportional to the intensity of the incident light (Beer-Lamart law). In other words the intensity of emitted light decreases exponentially as the thickness of the absorbing medium increase arithmetically. Application of the above principle to chemical analysis is that, monochromatic light beam usually emitted by deuterium lamp for ultra violet range and tungsten lamp for visible range along with quartz or silica cuvette of 1 cm path length are used. The blank /solvent is run before the analysis of the compound/exhibit. The wavelength at maximum absorbance i.e. λ-max of the compound obtained and pattern of the spectrum are the characteristics of that compound.

Limitation of this technique is the association or dissociation or ionization of the compound in the solvent. If the compound forms complexes the composition on which concentration depends changes and Beer-Lamart law is not valid for such cases. This technique is very useful for the analysis of a pure compound but may not be useful for the analysis of the mixture of the compounds. The derivative spectrophotometry may be used for the analysis of the mixture of the compounds where the second order spectrum gives an idea about the identity of the compounds in a mixture.

6. Infra-red spectrophotometry:

Infra-red spectrophotometry is study of reflected, absorbed, and transmitted radiant energy in the region of electromagnetic spectrum from wavenumber 125000 to 20/ cm. This electromagnetic spectrum is divided into three main regions;

1. wavenumber from 125000 to 4000/cm,

2. wavenumber from 4000 to 400/cm,

3. wavenumber from 400 to 20/cm.

The middle region is known as main IR region and may be used for the analysis of drug and pesticides. When the molecule of the compound is subjected to infra-red radiation's, transition takes place in rational and vibrational energy levels in ground state of the electron within the molecule. These transitions give rise to the absorption spectrum, which is the characteristic of the compound. This technique is also called finger print technique of the chemical identification. The advance version of this technique is FTIR spectrophotometry. Under the technique, a disc of potassium bromide along with solid sample is prepared under high pressure and IR spectrum is recorded after calibration of the equipment with polystyrene film. In case of liquid sample, cells that are used, having two parallel windows separated by spacing gasket and fitted with outlet and inlet ports. The reference spectrum of solvent is recorded before scannining of the sample.

7. Emission Spectrography:

This technique is one of the analytical techniques used for the elemental screening in the exhibit. It can be used for carrying out semi quantitative analysis of the inorganic elements. The technique is based on the emission property of the elements. In this technique the sample is made powder, mixed with carbon and filled in the graphite electrode. The filled electrode is arced at high temperature. The atom of the elements absorbed energy and get excited. The electron of the atoms jumps from lower enrgy level to higher energy level by absorbing the energy. When excited electron comes back to its lower states, it emits the energy in the form of line emission spectra. This spectra is recorded on a photography plate. After development of the plate the spectra can be seen in the form of various line at different wavelengths, which are the characteristc lines of the elements.

In this technique the element can be identified with out the use of standard sample. The lines are read with the help of comparator. The position / wavelength value of the elements are given in literature, which can be considered as the standard value of wavelength.

3. Inductively coupled Plasma-Atomic Emission Spectrometer (ICP-AES)

Atomic Emission Spectrometry (AES) is the oldest atomic spectrometric multi-elemental technique. All elements can be made to emit characteristic spectra under appropriate conditions. AES is divided into categories according to the source of energy that causes the element excitation. In general, the same source is used to atomize the sample and to excite (and possibly ionize) the atoms of the sample. The technique using classical sources (flame, arc and spark) arc widely applied. During the past quarter century, the plasma sources (new radiation source) have become commercially available and leading to lot of innovations for the revival of emission spectrometry.

Plasma is an electrically neutral, highly ionized gas composed of ions, electrons and neutral particles. The commonly used plasma have significantly higher gas termperatures and less reactive chemical environments then flames. The plasma gas is usually a stream of argon, which is energized with high frequency electromagnetic fields (r.f. or microwave energy) or with direct current when combined with a high quality spectrometers , plasma sources provide many of the characteristics of the ideal source. The r.f. inductively coupled plasma is to date the most frequenty-applied plasma. Samples are usually introduced into plasma in the same way as into a flame.

The ICP-AES technique is an excellent tool having many features like a high sensitivity towards most of the elements in the periodic table, speed, matrix effect correction, etc.

In ICP-AES technique, the sample solution is first aspirated by a nebuliser into micron size droplets before being introduced to the plasma where the sample is ionized and excited. The excited electrons re-emit the energy they have acquired in the form of electron magnetic radiation, which is composed of wavelengths characteristic of the elements emitting the radiation. The discrimination of these wavelengths is performed by an optical system which is then fed to a detector (photo

multiplier tube). The detector transforms the radiation into electrical signals that are captured by the data processing system.

Reference: Modern methods for trace elements determination by C.Vandecasteele and C.B.Black, John Wiley and Sons, UK, 1993, pg.139-143.

4. X Ray diffraction.

X ray diffraction is a versatile, non-destructive analytical technique for identification and quantitative determination of the various crystalline forms known as phases of compounds present in powdered and solid samples. The diffraction of x-rays by crystalline meaterials also helps in the study of internal defects in the atomic arrangement. The powder method applies to the study of polycrystalline materials whether they be in powder form, as supplied by nature or process or in compacted form as pellets or metallic mass.

A crystal lattice is a regular three-dimensional distribution (cubic, rhombic, etc.) of atoms in sparce. These are arranged so that they form a series of parallel planes separated from one another by a distance d, which varies according to the nature of material. For any crystal, planes exist in a number of different orientations- each with its own d-spacing.

When a monochromatic x-ray beam with wavelengh.λ is incident on a lattice planes in a crystal at an angle, x-ray diffraction occurs only when the distance traveled by the rays reflected from successive planes differs by a complete number n of wavelengths.

By varying the angle θ the Bragg's law conditions are satisfied by different d-spacing in polycrystalline materials.

(Bragg's law) $n\lambda = 2d \sin \theta$

Plotting the angular positions and intensities of the resultant diffraction peaks produces a pattern which is characteristic of the sample. Where a mixture of different phases is present, the diffractogram is formed by addition of the individual patterns.

Qualitative analysis

Every crystalline powder produces a characteristic diffraction pattern. This is the basis of qualitative analysis by powder diffraction. Identification is usually accomplished by the systematic comparison of an unknown pattern with a catalogue of standard data such as the Powder Diffraction File published by the International Center for Diffraction Data (ICDD).

Diffraction patterns of mixtures consist of the superimposed patterns of the individual components. There, powder diffraction is useful in analyzing mixtures as well as pure materials. As the number of components increases interpretation depends on the amount of prior knowledge available about the mixture.

Quantitative analysis

The x-ray diffraction pattern of a powdered mixture consists of the diffraction patterns of its constituent compounds. Each compound contributes its own pattern, with intensity proportional to the amount present in the mixture. Quantitative x-ray diffraction analysis consists of determining the amount of a compound from measurements of their corresponding intensity with that of the internal standards.

Advantages

An important advantage x-ray diffraction over other techniques is that the results obtained give information about the materials as they occur in the sample, rather than only about the individual elements or ions present. It is often the only satisfactory method of distinguishing amount polymorphs or detecting a compound in the presence of others containing the same elements.

Furthermore, the sample is not consumed in x-ray diffraction, so that it may be saved or subsequently analysed by other techniques. The term “non-destructive” frequently applied to x-ray analysis is not always appropriate here because crushing, grinding or other manipulations are frequently required to prepare the sample for powder diffraction.

Formation of committees at National Level for formulation of SOPs and Manuals:

Background: In view of technological advancements in the scientific arena, the Standard Operating Procedures (SOPs) and Working Procedure Manuals, around which the technical and analytical exercise takes place in the laboratory in the examination of crime exhibits, needs periodical review to keep the laboratory updated.

For uniform SOP/Manuals and reporting pattern in all the CFSLs / State FSLs following committees were formed by JS (PM), MHA by including members from Central and State FSLs in the following areas:

Discipline	CFSL Member	Member
Biology/DNA	Dr. A. K. Sharma, Director, CFSL, Kolkata/Guwahati	1. Sh. Arun Sharma, Director, FSL, HP 2. Sh. Srikumar, Director, Chemical Examiner Lab, Thiruvananthapuram.
Chemistry/ Narcotics	Sh. K. M. Varshney, Coordinator, CFSL, Pune	1. Dr. R. K. Gupta, Director, FSL, Chhattisgarh. 2. Sh. B Shanmukham, Director, FSL, Puducherry. 3. Dr. Harsh Sharma, Director, FSL, Sagar (MP)
Explosives	Dr. Sukhminder Kaur, Coordinator CFSL, Pune	1. One officer from FSL, Delhi 2. One officer from FSL, Maharashtra
Toxicology	Dr. Vimukti Chauhan, SSO, CFSL, Chandigarh	1. Dr. K. V. Kulkarni, Director, DFSL, Maharashtra 2. One officer from FSL, Karnataka.
Ballistics	Sh. S. S. Baisoya, CFSL Chandigarh	1. Dr. D. K. Kaushal, Director, FSL, Haryana 2. Sh. N. P. Waghmare, Director, FSL, Goa 3. Dr. S. S. Das, Director, FSL, Odisha
Documents	Sh. M. C. Joshi, Dy. Director, CFSL, Chandigarh (Shimla Unit) and Dr. S. Ahmad, DFSS HQs., New Delhi	1. Ms. Deepa Verma, Director, FSL, Delhi
Psychology, Computer, Audio-Video	Dr. S. K. Jain, Director, CFSL, Chandigarh and Sh. M. Krishna, AD, CFSL, Hyderabad	Officers from FSL: HP, Delhi, Gujarat and Maharashtra
Crime Scene	Dr. M. Baskar, Dy. Director (Physics), CFSL, Chandigarh	1. Dr. Harsh Sharma, Director, FSL (MP) 2. Sh. R. K. Gupta, Jt. Director, FSL, Chhattisgarh

The officers of CFSLs will coordinate with the experts of State FSLs for convening of meeting(s) in the state and Central FSLs and finalization of SOPs and manuals.