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Research Article

TECHNIQUES USED FOR BIOCHEMICAL INVESTIGATION IN RELATION TO FORENSIC ANALYSIS

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ABSTRACT

The aim of this review was to apply the knowledge & technology of science for the definition & enforcement of such laws. The forensic analysis is investigation the crime and examines material evidence. In forensic analysis various biochemical investigation techniques are used to examine the crimes like Hair analysis, Polygraphic test, serology test and finger print analysis. Several instruments are used in forensic analysis like IR, Chromatography, UV and Mass spectrophotometer. The characterization results showed that Forensic pharmacists engage in work relating to litigation, the regulatory process, or the criminal justice system.

Keywords: Polygraphic, "forensic", "forensis", "forensics".

INTRODUCTION

Forensic science is the application of a broad spectrum of sciences to answer questions of interest to the legal system. This may be in relation to a crime or to a civil action. The word "forensic" comes from the Latin adjective "forensis" meaning *of or before the forum*. During the time of the Romans, a criminal charge meant presenting the case before a group of public individuals in the forum. In modern use, the term "forensics" in place of "forensic science" can be considered incorrect as the term "forensic" is effectively a synonym for "legal evidence" or "related to courts". In a typical criminal investigation crime-scene investigators**,** sometimes known as scene-of-crime officers, will gather material evidence from the crime scene, victim and/or suspect. Forensic scientists will examine these materials to provide scientific evidence to assist in the investigation and court proceedings, and thus work closely with the police. Senior forensic scientists, who usually specialize in one or more of the key forensic disciplines, may be required to attend crime scenes or give evidence in court as impartial expert witnesses. [1]

SCOPE OF FORENSIC SCIENCE

- Forensic science is application of science to the law.
- Forensic science applies the knowledge & technology of science for the definition & enforcement of such laws.
- Forensic science is the application of science to those criminal and civil laws that are enforced by police agencies in a criminal justice system.

FORENSIC LABORATORY AND OFFICES IN INDIA

- Directorate of Forensic Science (DFS), Ministry of Home Affairs, Govt. of India
- Central Forensic Science Laboratory, C.B.I., New Delhi.
- Central Forensic Science Laboratory, Hyderabad in chemical science.
- Central Forensic Science Laboratory, Kolkata in biological science.
- Central Forensic Science Laboratory, Chandigarh in physical science. [2]

FORENSIC CHEMICAL ANALYSIS

Forensic chemical analysis has become important tools for solving crimes and assuring justice. Today, most forensic analysis techniques are qualitative and are used to identify or confirm the presence or absence of certain materials. However, in many cases applying quantitative analytical techniques can provide important additional information about material sources or the significance of material identifications. Project activities address measurement method and standards issues related to alcohol and drugs of abuse testing, crime scene investigations, chemical and biological weapons detection, and development of micro analytical devices enabling faster human identity testing. [3]

FORENSIC TOXICOLOGY

Forensic toxicology is a branch of Forensic Medicine dealing with Medical and Legal aspects of the harmful effects of chemicals on human beings. It is the use of toxicology and other disciplines such as analytical chemistry, pharmacology and clinical chemistry to aid medicolegal investigation of death, poisoning, and drug use. The primary concern for forensic toxicology is not the legal outcome of the toxicological investigation, but rather the technology and techniques for obtaining and interpreting the results.[4]

Post-Mortem Forensic Toxicology

which determines the absence or presence of drugs and their metabolites, chemicals such as ethanol and other volatile substances, carbon monoxide and other gases, metals, and other toxic chemicals in human fluids and tissues, and evaluates their role as a determinant or contributory factor in the cause and manner of death

Human-Performance Forensic Toxicology

which determines the absence or presence of ethanol and other drugs and chemicals in blood, breath or other appropriate specimen(s), and evaluates their role in modifying human performance or behavior; and

Forensic Urine Drug Testing

which determines the absence or presence of drugs and their metabolites in urine to demonstrate prior use or abuse.

INSTRUMENT USED IN FORENSIC ANALYSIS

In forensic analysis various type of instrument are used to determine the biochemical investigation of drug.

Thin-layer chromatography (TLC)

Thin layer chromatography is a widely used technique for the separation and identification of drug. It is equally applicable to drugs in their pure state, those extracted from pharmaceutical formulation, to illicitly manufactured materials and to biological samples.

Chromatography is used to separate mixtures of substances into their components. In the basic TLC experiment, the sample is applied to the layer as a spot or band near to the bottom edge of the layer. The separation is carried out in a closed chamber by either contacting the bottom edge of the layer with the mobile phase, which advances through the layer by capillary forces, or the mobile phase is forced to move through the layer at a controlled velocity by an external pressure source or centrifugal force.

A separation of the sample results from the different rates of migration of the sample components in the direction travelled by the mobile phase. After development and evaporation of the mobile phase, the sample components are separated in space, their position and quantity being determined by visual evaluation.

TLC is useful in detecting chemicals of forensic concern, including chemical weapons, explosives, and illicit drugs. Advances in TLC technology, largely driven by the efforts to quell terrorism, have benefited forensic science. [5]

Gas Chromatography (GC)

Gas chromatography (GC) is applicable to a wide range of compounds of interest to toxicologists, pharmaceutical and industrial chemists, environmentalists and clinicians. The separation is performed in a column (containing either a solid or liquid stationary phase) that has a continuous flow of mobile phase passing through it [usually an inert carrier gas, but more recently supercritical fluids (SCFs) have been used for some applications], maintained in a temperature–regulated oven. When a mixture of substances is injected at the inlet, each component partitions between the stationary phase and the gas phase as it is swept towards the detector. Molecules that have greater affinity for the stationary phase spend more time in that phase and consequently take longer to reach the detector. The detector produces a signal proportional to the amount of substance that passes through it, and this signal is processed and fed to an integrator or some other recording device. Identification of components was traditionally based primarily on peak retention time, but it is becoming increasingly more reliant on the nature of the response obtained from the detector. [6]

High Performance Liquid Chromatography (HPLC)

These techniques offered major improvements in speed, resolving power, detection, quantification, convenience and applicability to new sample types. The most notable of these modifications was high performance liquid chromatography (HPLC). It is also called as high pressure liquid chromatography since high pressure is used when compound to classical coloum chromatography

The systems used in chromatography are often described as belonging to one of four mechanistic types: adsorption, partition, ion exchange and size exclusion.

Adsorption chromatography arises from interactions between solutes and the surface of the solid stationary phase. Generally, the eluents used for adsorption chromatography are less polar than the stationary phases and such systems are described as 'normal phase'.

Partition chromatography involves a liquid stationary phase that is immiscible with the eluent and coated on an inert support. Partition systems can be normal phase (stationary phase more polar than eluent) or reversed–phase chromatography, referred to as RPC (stationary phase less polar than eluent).

Mass Spectrometer

Mass spectrometry is a technique used for measuring the molecular weight and determining the molecular formula of an organic compound. In an electron-impact mass spectrometer (EI-MS), a molecule is vaporized and ionized by bombardment with a beam of high-energy electrons. The energy of the electrons is ~ 1600 kcal (or 70 eV).Since it takes ~100 kcal of energy to cleave a typical s bond, 1600 kcal is an enormous amount of energy to come into contact with a molecule. Usually only a portion of this energy is transferred to the molecule. The electron beam ionizes the molecule by causing it to eject an electron. Analogously, a mass spectrometer contains an ion source that generates ions, a mass analyzer that separates the ions according to their mass-to-charge ratio, and an ion detector.

Ultraviolet and visible spectrophotometer.

Analytical absorption spectroscopy in the ultraviolet (UV) and visible regions of the electromagnetic spectrum has been widely used in pharmaceutical and biomedical analysis for quantitative purposes and, with certain limitations, for the characterisation of drugs, impurities, metabolites and related substances. By contrast, luminescence methods, and fluorescence spectroscopy in particular, have been less widely exploited, despite the undoubted advantages of greater specificity and sensitivity commonly observed for fluorescent species. However, the wider availability of spectrofluorimeters able to present corrected excitation and emission spectra, coupled with the fact that reliable fluorogenic reactions permit non–fluorescent species to be examined fluorimetrically, has led to a renaissance of interest in fluorimetric methods in biomedical analysis. Molecular absorption in the UV and visible region arises from energy transitions that involve the outer orbital or valency electrons. Spectra in liquid media are usually broad, relatively featureless bands, a result of the large number of closely spaced vibrational and rotational transitions.

Infra-Red Spectroscopy

Infra–red (IR) spectroscopy is the study of the scattering, reflection, absorption or transmission of IR radiation in the spectral range 800 nm to 1000000 nm (0.8 to 1000 μm). IR radiation can excite molecular vibrations (and associated molecular rotations). At room temperature, a molecule is generally in its ground electronic state where it sits in its ground vibrational state. Provided the incoming IR radiation has the appropriate energy (wavelength, wavenumber), resonant absorption occurs to excite the molecule to a particular higher vibrational state. Vibrational transitions give rise to an absorption spectrum characteristic of the compound. A major advantage of IR spectroscopy is the ability to measure relatively heterogeneous materials and poorly characterized samples, particularly in condensed phases.

Immunoassay

Immunoassays have a firm place among routine methods for the analysis of drugs in biological fluids and other matrices. The technique may be used by the smallest or largest of laboratories with methods that range from single–use point–of–care tests for the analysis of a single sample to fully automated systems capable of analyzing thousands of samples per day. The drug immunoassay involves setting up a competition for binding to the antibody between antigen (drug) in the sample and a fixed amount of antigen added as part of the test system. Forensic toxicology encompasses the determination of the presence and concentration of drugs, other xenobiotics and their metabolites in physiological fluids and organs and the interpretation of these findings as they may impact on legal issues. These include medical examiner investigations, driving under the influence and other transportation accident investigations, workplace pre-employment, random and for-cause drug testing and judicial monitoring of arrestees and parolees.

METHODS FOR ANALYSIS IN FORENSIC ANALYSIS

Hair Analysis

Hair analysis was used to evaluate exposure to toxic heavy metals, such as arsenic, lead, mercury. This was achieved using atomic absorption spectroscopy which allowed detection in the nanogram range. Gas chromatography-Mass chromatography is method of choice for hair analysis. [6]

Pubic hair, arm hair & axillary hair have been suggested as an alternative source for drug detection when scalp hair is not available.

Mechanism of drug incorporated into hair

It is generally accepted that drug can enter into hair by two processes- Adsorption from external environment and Incorporation into the growing hair shaft from blood that supplies the hair follicle.

DRUG ANALYSIS

The analysis of morphine in hair to determine the history of opiate abuse reported the use of radioactive immunoassay & gas chromatography.

Radioactive immunoassay is the common screening test for hair. It can be used without any modification at pH values above 7. The radioactive immunoassay result should be confirmed by Gas chromatography-Mass chromatography.

Chromatography methods have been used as screening and confirming test they allow quantification of the drug & drug metabolites.

TLC – Morphine.
HPLC – Morphine, Haloperidol.
GC – Afetamines or Cocaine.

EXAMPLE: - COCAINE

Procedure for detection of Cocaine

Decontamination- Take a hair strand (100 mg). Wash with 5ml methylene chloride for 2 minutes. Dry with adsorbent paper. Second wash in 5ml methylene chloride for 2 minutes.

Homogenisation –Pulverise the hair in a ball mill for 10 minutes at 100 cycles/min.

Solublisation –To 30 to 50 mg of powdered hair add 1 ml 0.1 M Hcl & 200 mg of deuterated opiate & cocaine derivatives. Incubate for 16 hours at 56 C.

Extraction – Homogenate with 10 ml chloroform: propan-1-ol: hept-1-ane (50:17:33 v/v/v). Agitation 20 min. at 95 cycles/min. Centrifuge for 15 min. at 3000 rpm. Purify the organic phase by acid extraction (5ml 0.2M Hcl) then alkaline back-extraction (1ml 1M NaOH + 2ml phosphate buffer ph 8.4 in chloroform). Collect organic phase and evaporate to dryness.

Derivatisation –To the dry extract add 30 ul N,O-bis(trimethyl silyl)trifluroacetamide.(BSTFA)+ 1% trimethylchlorosilane(TMCS). Incubate for 30 min. at 70 C.

Analysis – A 1.5 ul portion is injected in splits mode into an HP5-MS capillary column (3m x 0.25 mm). The GC parameter: - Flow rate helium N55 1.0 ml/min. Injector temperature- 270 C.

Polygraphic Test

A polygraph (popularly referred to as a lie detector) is an instrument that measures and records several physiological responses such as blood pressure, pulse, respiration, breathing rhythms, body temperature and skin conductivity while the subject is asked and answers a series of questions, on the theory that false answers will produce distinctive measurements. The polygraph measures physiological changes caused by the sympathetic nervous system during questioning. Within the US federal government, a polygraph examination is also referred to as a psychophysiological detection of deception (PDD) examination.[7]

Polygraphs are in some countries used as an interrogation tool with criminal suspects or candidates for sensitive public or private sector employment. The use and effectiveness of the polygraph is controversial, with the manner of its use and its validity subject to ongoing criticism.

TESTING PROCEDURE

Polygraph examiners use two types of instrumentation: analog and computerized. At that time the most commnly used is computerized instrumentation.

A typical polygraph test starts with a pre-test interview to gain some preliminary information which will later be used for "Control Questions", or CQ. Then the tester will explain how the polygraph is supposed to work, emphasizing that it can detect lies and that it is important to answer truthfully. Then a "stim test" is often conducted: the subject is asked to deliberately lie and then the tester reports that he was able to detect this lie. Then the actual test starts. Some of the questions asked are "Irrelevant" or IR ("Is your name ramesh kumar?"), others are "probable-lie" Control Questions that most people will lie about ("Have you ever stolen money?") and the remainders are the "Relevant Questions ", or RQ, that the tester is really interested in. The different types of questions alternate. The test is passed if the physiological responses during the probable-lie control questions (CQ) are larger than those during the relevant questions (RQ). If this is not the case, the tester attempts to elicit admissions during a post-test interview, for example, "Your situation will only get worse if we don't clear this up"

Serology Test

In Serology test various types of analysis occurs like blood sample analysis, paternity tests and semen analysis. [8]

In blood sample analysis the Luminol test - a spray that produces a faint glow in total darkness - better for older stains and Phenolphthalein (Kastle-Meyer color test) - when mixed with hydrogen peroxide and blood turns deep pink - highly sensitive

In Paternity tests Accurate 90% - type ABO than rather antigens of system complex a identifies test (HLA) antigen leukocyte human. Hepatoglobin blood typing - 95% accurate. DNA testing - 95% accurate

In Semen analysis Microscopic examination may reveal spermatozoa - brittle when dry and disintegrates easily. Chemical test - acid phosphatase color test (acid phosphatase is secreted by prostate gland). The suspected stain is rubbed with a piece of water-moistened filter paper. A drop or two of test solution - an acidic solution of sodium alpha-naphthylphosphate and the dye Fast Blue B - are added. A purple color develops if phosphatase is present - false test possible with some fungi, contraceptive creams and vaginal secretions BUT none develop as fast as seminal fluid.

Fingerprints Analysis

According to criminal investigators, fingerprints follow 3 fundamental principles

A fingerprint is an individual characteristic; no two people have been found with the exact same fingerprint pattern. A fingerprint pattern will remain unchanged for the life of an individual; however, the print itself may change due to permanent scars and skin diseases. Fingerprints have general characteristic ridge patterns that allow them to be systematically identified. [9]

There are 3 specific classes for all fingerprints based upon their visual pattern: arches, loops, and whorls.

Fingerprint Factoid: 60% of people have loops
35% have whorls
5% have arches

ARCHES

Arches are the simplest type of fingerprints that are formed by ridges that enter on one side of the print and exit on the other. No deltas are present.

LOOPS

Loops must have one delta and one or more ridges that enter and leave on the same side. These patterns are named for their positions related to the radius and ulna bones

WHORL

Whorls have at least one ridge that makes (or tends to make) a complete circuit. They also have at least two deltas. If a print has more than two deltas, it is most likely an accidental.

Trace Evidence The term trace evidence refers to a very broad category of physical evidence. The trace evidence category covers a larger variety of physical evidence types than any other division. Two very different notions contribute to concept of trace evidence. One notion is related to size. Small amounts of material may be referred to as traces, and evidence of concern in forensic investigations is often very small. Here the notion of trace seems appropriate, but size is not the defining characteristic. There are various types of traces:-Hair, Fibers, Soil, Glass and Paint. [8]

ROLE OF FORENSIC ANALYSIS

Forensic Science can be defined as criminalistic science. In other words the scientific studies or investigation of crime can be termed as Forensic Science. Along with the development of science and technology the pattern of our society has also changed to cope with the day to day development. According the criminal also often uses different techniques for commission of various crimes within our society. So it has become a problem for the law enforcing agencies to check the potentiality of crimes. For such checking the need of forensic science becomes an essential prerequisite on the part of the investigative agencies. [10]

- The operation of forensic science is nothing but the application of techniques and methods of basic science techniques and methods of basic science for various analyses of exhibits associated with crimes. Since it's beginning the scientists of the Forensic Science Laboratory, Assam have been rendering invaluable service to the investigating agencies in various ways for the cause of justice.
- The scientific examination of a forensic scientist adjoins a missing link or strengthens a weekly in the chain of investigation by furnishing an impartial and establishes evidence, thereby helping the court to come to a conclusion regarding the criminals and their punishments. The field of study or examination of forensic scientist is very wide, diversible and unpredictable.
- Generally the duties and responsibilities of forensic scientist are very hazardous, onerous and risk bearing. Because they are to deal with the material exhibits pertaining to various nature of crimes such as murder, rape, blood, saliva, firearms, ammunitions, explosives, and explosives substances, liquor, hashish, opium, adulterated petrol, kerosene, diesel, etc. and other chemical vehicles involved in accidents, various types of paints.
- Weapons used in burglary, arson, etc. different types of poisons and poisons and poisonous substances, hair, skeletal remains and other plant or animal remnants. Apart from these, forensic scientists are also to examine the forged signatures and documents along with the photographic analysis of all materials exhibits. Any material exhibit encountered in the way of investigation needs to be thoroughly examined to prove or disprove its association a particular crime or criminal.
- The forensic scientist are to examine the material exhibits connected with various nature of crimes covering all sections of I. P. C. and other relevant acts and laws of the land. Unlike other research and analytical materials, forensic scientists are required to work with limited quantity and amount of materials generally left behind or carried away by criminals.
- For better collection of exhibits for various range of studies, forensic scientist are often summoned to the scene of crime so as to assist the investigation agencies in determining clue by means of scientific analysis.

Role of pharmacist in forensic analysis

Pharmacy is the science of medications. Forensic means the application of science to legal issues. Despite the common misconception, the word & forensic does not mean death. Therefore, forensic pharmacy is the application of medication sciences to legal issues. Forensic pharmacists engage in professional work relating to litigation, the regulatory process, or the criminal justice system. Forensic pharmacists may testify in court, engage in regulatory activities, consult for lawyers or law enforcement officials, or perform other medico-legal related work.

Forensic pharmacists are involved with administrative hearings and civil and criminal trials. A few pharmacists are full-time forensic pharmacists who work for the government (e.g. Food and Drug Administration (FDA), Drug Enforcement Administration (DEA), state regulatory agencies, etc.) or work as forensic toxicologists. Most forensic pharmacists are part-time consultants while working full-time in community pharmacies or hospitals, conducting research, teaching, or clinical consulting. These part-time forensic pharmacists may consult for lawyers, law enforcement, or serve on drug testing crews for athletic teams.

Forensic pharmacists engage in work relating to litigation, the regulatory process, or the criminal justice system. Forensic pharmacy overlaps with many other forensic fields. Pharmacists hold a variety of positions with local, state, and federal governments. Many pharmacists do freelance work as forensic litigation consultants. A forensic pharmacist can be a valuable resource in legal cases relating to malpractice, adverse drug reactions, and drunk and drugged driving, and numerous other types of civil and criminal cases. There are no specific training programs or certifications for forensic pharmacists. However, certification in clinical areas does enhance the status of an expert.

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