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Analytical Techniques in Forensic Science: MS & Bio-Sensor based diagnostics

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ABSTRACT

Background/Objectives: Forensic science is a stream which owes its growth to the advancements in technology. Forensic science analyses samples or material evidences like blood, skin, semen, hair collected from the site of crime through detection techniques. **Methods:** Various analytical techniques including Gas/Liquid Chromatography, HPLC, Mass Spectrometry, Immunoassays, enzymatic assays, DNA profiling techniques, PCR, biosensing and metabolomics involved for forensic studies. LC-MS is efficient in telling the chemical nature of almost all kind of samples. DNA profiling provides the fingerprint of individual's DNA which are used in criminal identification and solving disputes related to paternity and maternity. PCR allows gaining great deal of information from very limited amount of samples. **Conclusion:** Cumulative use of the detection techniques has totally changed the parameters of forensic analysis. The traces of samples can easily be analysed accurately and efficiently. This review provides an overview of the recent methods and technology used for forensic analysis.

Keywords: Forensic analysis, LC-MS, Biosensors, Metabolomics, DNA profiling.

INTRODUCTION

Forensic science is the application of a broad spectrum of sciences to answer the questions of interest to a legal system [1].It is the science which is totally based on other disciples of science like molecular biology, biochemistry, biotechnology, immunology etc. for its working. It collectively uses the principles of basic sciences to reach to a final result and clears the ambiguity of situations. Developments in forensics have dramatically changed the picture of crime investigations. It applies the knowledge of basic science to criminal and civil laws which aids in completing the investigation and court proceedings. Forensic science adjoins the missing links of investigation by analysing evidences and helps the court to come to its final verdict [2]. Forensic science deals with variety of sample types depending on nature of crimes such as murder, rape, blood, saliva, firearms, ammunitions, explosives, and their after-blast residues, alcohol, drugs, adulterated petrol, kerosene, diesel, etc. and accident's residues. It uses traces of evidences found on crime scene. Traditionally forensic science has to rely on canine olfaction for narcotic and explosive investigations [3]. But presently along with conventional methods more innovative methods are practiced to gain more accurate results. Various investigative techniques like Gas/Liquid Chromatography, HPLC, Mass Spectrometry, Immunoassays, enzymatic assays, DNA profiling techniques, PCR, bio sensing, metabolomics are used in forensic laboratories which have enhanced the sensitivity and accuracy of analysis. Automation of techniques have now revolutionised the analytical studies. Technology has helped in providing not just the robust but also reliable results.

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ENZYMATIC METHODS

Concentration of alcohol in saliva is almost equal to its concentration in blood. Considering this fact saliva is used for determining blood alcohol concentration (BAC). Alcohol oxidase (ALOx) is highly specific for alcohol (EtOH). The Instant Alcohol Saliva Test Strip has also been fabricated for alcohol testing. It provides faster and efficient results. Whenever the BAC is more than 0.02% the strip confirms the presence of alcohol in the blood [4].

The acid phosphatase colour test is used to detect the presence of semen. Amount of the acid phosphatase enzyme in semen is nearly 1000 times greater than any other body fluid. Acid phosphatase is secreted by prostate gland. Semen can be found on clothes, in vaginal fluids or nearby objects of crime scene. Stain of semen is moistened with water and a drop of sodium alpha-naphthylphosphate and the dye Fast Blue B - are added on it. Appearance of purple colour confirms the presence of acid phosphatase. No other body fluid develops colour so fast that is why chances of false positive results are very rare. Assay for prostatic acid phosphatase might be used as a more specific test for the identification of semen in samples from cases of sexual assault [5].

IMMUNOASSAYS

Immunoassays are a more reliable and well-studied mode of diagnosis in clinical and forensics. Presence and concentration of surface antigens of pathogens, cells are detected using immunoassay in laboratories. Forensic toxicology encompasses the determination of the presence and concentration of drugs, other xenobiotics and their metabolites in body fluids, organs and the interpretation of these findings may impact on legal issues. Most of the drug immunoassays are competitive. It 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. Once the Ab gets bound with the Ag, signal is produced. In Homogeneous immunoassays original sample remain bound to Ab. Enzyme immunoassay (EIA) and fluorescent polarization immunoassay (FPIA) are done to analyse the results of assay. In EIA enzyme activity decreases when drug is bound, in FPIA emission in a polar field increases when sample is bound and kinetic interaction of Ag-Ab in solution (KIMS) immunoassay lattice formation is inhibited due to the presence of sample Ag. Homologous immunoassays can be easily automated. In heterogeneous immunoassays unbound sample is removed before signal measurement. Heterologous immunoassays include radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) [6]. Procalcitonin (PCT) is considered as a marker for inflammatory response due to sepsis and the same can be used in forensics as parameter to determine the death due to sepsis. Immunoluminometric assay where Abs specific for PCT are labelled with luminescent acridine derivative is performed to quantify the level of PCT [7]. ELISA can also be used for detection of semen glycoprotein p30. Semen for normal and vasectomized men contains sufficient amount of p30 (1.55mg/ml of seminal fluid) [8]. Detection limit of assay, cross-reactivity of antibodies and chances for interference poses a break in the success of immunoassays.

CHROMATOGRAPHIC TECHIQUES

1.Thin layer chromatography (TLC):

In forensic labs TLC is used for the identification and comparison of drugs, explosives, inks and dyes. Only requirement of TLC is that the sample must be soluble in mobile phase. Chromatography is method of separation using two phases; a mobile phase and a stationary phase. In TLC, the stationary phase is a thin layer of alumina or silica on a plate. The mobile phase depends on the nature of the sample to be separated. Spot of sample is applied just at the edge of bottom and the solvent is allowed to run through the plate via capillary action and removed before the solvent reaches the top. Separation of the chemicals occurs due to their differential rate of migration and solubility in mobile phase. The point at which the solvent has reached on the plate is marked. After development and evaporation of mobile phase, the path travelled by each spot is measured which is called Retardation factor (R_f) and compared with the R_f of standard chemicals [9]. TLC of drugs not just provides detect the presence of drug but also tells about the nature of drug. It is also useful in analysing chemical weapons and explosives [10]. Only limitation of TLC is the solubility of sample as the ink of gel pen is insoluble, so it gets quite difficult to analyse those samples. HPTLC has drastically changed the parameters of TLC as the sample requirement has been reduced to Nano grams, size of strip has been reduced to 3-4 inches and it is also saves time.

2. Gas chromatography (GC):

GC is considered as a mature analytical technique in forensic science. Its high sensitivity, selectivity, resolution and speed, good accuracy has made it an indispensable tool of forensic science. GC is applied for the analysis of a broad range of volatile and semi volatile compounds. As the mobile phase in GC is usually an inert gas then only a mixture of volatile organic compounds can partition itself between stationary and mobile phase. Molecules having higher affinity for stationary phase take more time to separate while molecules which partitions more in carrier gar separates faster. Traditionally identification of compounds was done on the basis of retention time, but now-a-days it is becoming increasingly more reliant on the nature of the response obtained from the detector [11].Multidimensional gas chromatography (GC × GC) and fast gas chromatography have contributed significantly in analysing to a wide range of compounds of interest to toxicologists, pharmaceutical and industrial chemists[12].GC/MS provides confirmatory results for the presence of drugs and metabolites in forensic urine drug testing.

3. High-performance liquid chromatography (HPLC):

HPLC is also known as high-pressure liquid chromatography as high pressure is applied to the classical chromatographic columns. HPLC is also based on the partitioning of compounds in stationary and mobile phase. The systems used in chromatography are often one of four mechanistic types: adsorption, partition, ion exchange and size exclusion. But it offers high resolving power, lower detection limit, and faster results with more convenience. HPLC is more efficient because of the kind of detectors used to analyse the eluting sample. Refractive index detector (RI) is a universal detector as it responds to all compounds that elutes, but lower detection limit is a drawback. Whereas UV/VIS detectors and UV/VIS spectrophotometers responds to a wide variety of compounds of forensic interest with good to excellent sensitivity. Mass spectrometer (MS) provides quantitative as well as qualitative information data of almost all compounds. But its high cost is a limitation to its use in routine analysis. In evidence analysis HPLC can analyse the presence of drugs in body fluids, organic extractions of soil, explosives, inks, dyes and plastics[13].

MASS SPECTROMETRY

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 electron beam ionizes the molecule by causing it to eject an electron and mass analyser that separates the ions according to their mass-to-charge ratio, and an ion detector. Coupling of chromatographic techniques with mass spectrometry has brought a significant breakthrough in analytical toxicology. LC-MS (Liquid chromatography-Mass spectrometry) is relevant for the screening, identification and quantification of drugs, poisons and their metabolites in

blood, urine and serum [14]. Systematic toxicological analysis using LC-MS is done through following techniques:

1. Targeted screening using liquid chromatography–tandem mass spectrometry:
This method is used to analysis a set of known or expected compounds. It has been used for analysing for single or multiple drug classes through LC-multiple (or selected) reaction monitoring (MRM or SRM). 238 drugs have been identified in a blood sample using targeted screening[15]. Screening of analyte is limited to 300 in targeted screening but specificity of this method is incredible[16].

2. Liquid chromatography–mass spectrometry with reference libraries:
Reference libraries contain structural information of variety of chemicals. Reference libraries are generated by collision-induced dissociation (CID). Data is acquired as single or multiple mass spectra and compared with standard techniques like GC or HPLC. Reference library for more than 500 toxicologically relevant compounds has been created by LC–MS CID based on multiple spectral entries [17]. Variability of spectra between instruments is the limitation to the routine use of LC-MS in chemical analysis of sample.

3. Liquid chromatography–(tandem) mass spectrometry with exact mass:
TOF-MS can determine the mass of a compound upto four decimal places. The ability to determine the *m*/*z* of an ion to within 5 parts per million (ppm) allows the determination of a unique ionic formula of the compound. Mass analysis is not sufficient to characterise a compound so CID fragmentation patterns from Reference libraries are consulted to obtain full analysis.

Analgesics, sedative-hypnotics, benzodiazepines, antidepressants, ephedrine, anti-diabetics, toxic alkaloids, quaternary ammonium drugs and various other drugs can be screened using LC-MS. It has also been used to investigate Chemical warfare agents and their metabolites like soman, sarin, sulphur mustard etc. LC-MS is also a well-established technique for analysing high explosives like RDX, TNT, HMX and their post-blast residues. LC-MS has emerged as a totally versatile, reliable and sensitive tool.

MOLECULAR TECHNIQUES
DNA of an individual has always a unique sequence of base pairs. 'DNA fingerprint' has always a characteristic that no two individuals will have same DNA fingerprint. Although 99% of DNA is similar in all individuals which code for basic body proteins but 1% sequences are always different for two different individuals. Variation in DNA is due the presence of VNTRs (Variable number of tandem repeats). DNA based technique unlock new realm in forensics. Sample for analysis can be obtained from samples like blood, semen, saliva found on the site of crime.

1. DNA profiling:
DNA profiling or DNA fingerprinting studies the characteristic genetic profiles of individuals to solve parental disputes and other criminal investigations. DNA fingerprint of an individual is derived by three modes: short tandem repeat (STR), Single nucleotide polymorphism (SNP) and Restriction Fragment Length Polymorphism (RFLP).

A. Short tandem repeat (STR):
STR is a sequence of DNA which consists of adjacent repeats of a set of nucleotides. The STRs are the most informative genetic markers used for DNA profiling. STRs consist of 70-250bps stretches of DNA. Polymorphism occurs when homologous STR loci differ in the number of repeats between two different individuals. By identifying repeats of a particular sequence at specific locations in the genome, it is possible to create a genetic profile of an individual. The DNA extracted from the cells of a forensic sample is amplified in specific regions through PCR. Then the amplified sequence is electrophoresed. After electrophoresis characteristic pattern of the unique genetic/DNA profile of the suspect will be generated and this can be used for further investigations[18]. STR analysis is the most common and reliable method of generating genetic profiles in forensic science. Even in highly degraded DNA where DNA of long lengths can't be generated, STRs play very significant to analyse polymorphism. There are currently over 10,000 published STR sequences in the human genome. Researchers are trying to convert STRs into mini STRs, which can be accommodated into one multiplex analysis and STR kits be reconfigured into mini-STR kits for routine analysis of forensic evidences[19].

B. Single nucleotide polymorphism (SNP):
SNP is another genetic marker used for DNA profiling. SNPs are responsible for ~ 85% of human genomic variation. They are much shorter DNA sequences of around 50bps and more abundant as compared to STRs. Not all the SNPs are significant in DNA profiling. There are mainly four classes of SNPs:

(i) Identity-testing SNPs help in determining the individual's identity as they are relatively more heterozygous in every individual and shows low heterogeneity in population. These SNPs are more relevant tool in forensic molecular biology because of their widest applications. They can aid in analysing highly degraded DNA. Extensive research is going on to select the more specific and highly polymorphic core identity-testing SNPs to diversify their applications.

(ii) Lineage-informative SNPs, sets of tightly-linked SNPs markers which help in identify missing persons through kinship analyses. These SNPs analyses the genetic markers residing on the maternally-inherited mitochondrial genome and the paternally-inherited Y chromosome to obtain the kinship.

(iii) Ancestry-informative SNPs for establishing high probability of an individual's biogeographical ancestry to indirectly infer some phenotypic characteristics for investigative lead value, requiring low heterozygosity and high population heterogeneity.

(iv) Phenotype-informative SNPs tells about the phenotype of the

person. Particular phenotypic characteristic such as skin color, hair color, or eye color are very informative in investigations where there is no suspect. One some characteristic phenotypic trait like the height of individual or the facial restructuring can point out the accused and further confirmation can be obtained by getting the DNA profile of the suspect[20]. Restriction fragment length polymorphism (RFLP): RFLP is based on the use of different sequence specific restriction enzymes and formation of different sized fragments. The DNA obtained from forensic sample is cut with restriction enzymes and where ever in the genome the R.E. find its specific sequence it creates a cut. Then the fragments are separated by electrophoresis. DNA of two different individuals will result in formation of two different electrophoresis patterns due to the variation in the sequences of DNA. Differently sized restriction fragments are result of polymorphism in individuals [21].

POLYMERASE CHAIN REACTION (PCR)

PCR was developed by Karry Mullis in 1983. Now, it is well known that PCR is a very fast method of DNA amplification. In forensic science where we just have traces of sample for analysis, in that situation PCR is a boon. Robust DNA amplification is very significant when touch DNA or Low Copy Number (LCN) DNA is available. When the sample is obtained from swabs and the amount of DNA is very less in that situation PCR makes millions of copies of DNA in few minutes using very few copies of template DNA, polymerase enzyme, oligonucleotides and short primers specific to the sequence to be amplified. To enhance the sensitivity of analysis number of PCR cycles is increased, PCR volume is reduced and post-PCR clean up step is implemented to concentrate the sample for analysis and competitive ions are removed[22]. Contaminants present in forensic samples can act as inhibitors to DNA amplification and lead to stochastic amplification. Presence of inhibitors reduces the number of templates. To diminish the effect of inhibitors additives like BSA are added which counteract the effect of inhibitors PCR-based methods are now coupled with automated fluorescent detection technologies to improve the sensitivity of analysis[23].

BIOSENSORS

A biosensor is a device that uses biological materials to detect and monitor the presence of specific chemicals in an area. They have potential to detect the presence of chemicals calorimetrically, colorimetrically, electrochemically and optically. DNA biosensors use DNA probes to hybridise with sample DNA. These biosensors can be used to diagnose genetic disorders and also plays crucial role in forensic sciences. PCR amplification can also be hyphened with DNA biosensor to detect trace amount of DNA on crime scene [24]. After getting a clue of genetic disorder it get easier to find the accused out of random suspects. Further confirmation can be obtained by the DNA profiling of the individual. Prostate Specific Antigen (PSA) biosensors are a tool of choice in the cases of sexual assault where sperms are absent. PSA is also a component of semen, that's why act as a forensic marker. Presence of PSA in sample confirms the sexual assault. PSA Biosensor is a miniaturised device also suitable for on-site monitoring. It has high specificity and sensitivity[25].

METABOLOMICS

Metabolomics is the latest branch of analytical biochemistry. Metabolomics is the study of metabolome (entire collection of metabolites of a cell under given set of conditions). Metabolite can be a peptide, oligonucleotide, lipid, alcohol, ketone, sugar, amine, alkaloid, steroid, drugs or xenobiotic [26]. Data that remain unrevealed by proteomics and genomics is analysed with the help of metabolomics. Metabolite profiling helps in identification of metabolites as the analysis is based on their spectral peaks and calibration curves. It is involved in toxicology testing, drug compliance, genetic disorder tests, drug phenotyping, and blood analysis [27,28].

Significant efficiency of metabolomics is because it uses more than one method for analysis like NMR or MS fingerprinting, GC/Ms, LC/MS. Statistical tools like XCMS[29],MetAlign[30] MZmine [31] and MathDAMP [32] are also available for the analysis of data obtained from mass spectrometry.

CONCLUSION

Continuous developments in the fields of molecular biology and biophysical techniques have opened new doors to forensic science. LC-MS has emerged as a very versatile, sensitive and robust technique for analysis of chemicals. UPLC (Ultra performance Liquid Chromatography) has evolved as a much faster chromatographic technique. With the aid of PCR traces of DNA have proved significant for forensic analysis. Even highly degraded DNA can be analysed and generate genomic profile of accused. But still there are a number of loop holes in forensics which need to be addressed for better analysis. Biosensors are still a taboo in forensic and their full potential has to be explored. Also perfectly trained lab personnel are required to study the results more efficiently. As we all know analytical science is very demanding, it keep on demanding to improve the sensitivity and speed of analytical methods.

REFERENCES

1. Ricciuti E. Science 101: forensics, HarperCollins Publishers, New York, USA (2007).
2. Mehta P, Dashora A, Sahu D, Garg RK, Agrawal P, Joshi B, Sharma D. Techniques used for biochemical investigation in relation to forensic analysis. Innovare Journal of Life Science , 2, 2013, 7-10.
3. Frederickx C, Verheggen FJ, Haubruge E. Biosensors in forensic sciences. Biotechnol. Agron. Soc. Environ., 15, 2011, 449-458.
4. www.drugcheck.com/_images/DC172-B_ALCSaliva-strip_insert.pdf
5. Fishman WH, Dart RM, Bonner CD, Leadbetter WF, Lerner F, Homburger F. A new method for estimation of serum acid phosphatase of prostratic origin applied to the clinical investigation of cancer of prostrate. J Clin Invest., 32, 1953, 1034-1044.
6. Moody DE. Immunoassays in Forensic Toxicology: Encyclopedia of Analytical Chemistry, 2006.
7. Tsokos M, Reichelt U, Nierhaus A, Puschel K. Serum procacitonin (PCT): a valuable biochemical parameter for

the post-mortem diagnosis of sepsis. Int. J Legal Med., 114, 2001, 237-243.
8. Howard CB, Graves MD, M.P.H., Sensabaugh GF, Crim D, Blake ET. Postcoital Detection of a male-specific Semen Protein-Application in Investigation of Rape, N Engl J Med., 312,1985, 338-343.
9. Clugston MJ. Dictionary of Science Penguin, 2nd Edition.
10. Krupadanam GLD, Prasad DV, Reddy KLN. "Analytical chemistry" Universities press; 1st edition (2001); pp. 69-72.
11. Moffat AC, Osselton MD, Widdop B. Clarke's Analysis of Drugs and Poisons. Pharmaceutical press, 3rd edition, pp.102, 127-129, 303, 315, 329, 382, 395, 428, 505.
12. Kabir A, Furton KG. Applications of Gas Chromatography in Forensic Science In:Gas Chromatography, 2012, pp.563–604
13. Houck MM, ý Siegel JA .Fundamentals of Forensic science – 2010, pp.142-145.
14. Wood M, Laloup M, Samync N, Fernandez MMR, Bruijn EA, Maesb RAA, Boeck GD. Recent applications of liquid chromatography–mass spectrometry in forensic science. Journal of Chromatography A, 113, 2006, 3–15.
15. Gergov M, Ojanper I, Vuori E. J. Chromatogr. B, 795, 2003, 41.
16. Mueller CA, Weinmann A, Dresen S, Schreiber A, Gergov M. Rapid Commun. Mass Spectrom, 19 ,2005, 1332.
17. Humbert L, Grisel F, Bondoux G, Lhermitte M. Presented at the Annual Meeting of the International Association of Forensic Toxicologists (TIAFT), Paris, 27–30 August 2002.
18. Butler JM. Forensic DNA Typing: Biology,Technology, and Genetics of STR Markers, 2nd Edition, Academic Press, 2005.
19. Mulero, J.J., Chang CW, Lagace RE, Wang DY, Bas JL, McMahon TP, Hennessy LN. Development and validation of the AmpFlSTR MiniFiler PCR Amplification Kit: a MiniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA. J. Forensic Sci., 53, 2008, 838-852.
20. Budowle B, Daal AV. Forensically relevant SNP classes. BioTechniques, 44, 2008, 603-610.
21. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet., 32, 1980, 314-331.
22. Budowle B, Hobson DL, Smerick JB, Smith JAL. Low copy number: consideration and caution, Proceedings from the Twelfth International Symposium on Human Identification 2001, pp. 1-9. Promega Corporation, Madison.
23. Hochmeister, M.N., Budowle B, Borer UV, Eggmann UT, Comey CT, Dirnhofer R. Typing of DNA extracted from compact bone tissue from human remains. J. Forensic Sci., 36, 1991,1649-1661.
24. Yongkang Ye, Huangjian Ju. DNA electrochemical behaviours, recognition and sensing by combining with PCR techniques. Sensors, 3, 2003, 128-145.
25. Healy DA, Hayes CJ, Leonard P, McKenna L, Kennedy RO. Biosensor developments: application of prostate-specific antigen detection. Trends in Biotechnology, 25, 2005, 125-131.
26. Gahlaut A, Vikas, Dahiya M, Gothwal A, Kulharia M, Chhillar AK, Hooda V, Dabur R. Proteomics & metabolomics: Mapping biochemical Regulations. Drug invention today, 5, 2013, 321-326.
27. Glassbrook N, Ryals J. A systematic approach to biochemical profiling. Curr. Opin. Plant Biol., 4, 2001,186-190.
28. Harrigan GG, Goodacre R. Metabolic Profiling: Its Role in Biomarker Discovery and Gene Function Analysis. Boston: Kluwer Academic Publishers; 2003.
29. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem. 78, 2006, 779-787
30. Katajamaa M, Miettinen J, Oresic M. MZmine: toolbox for processing and visualization of mass spectrometry based molecular profile data. Bioinformatics. 22, 2006, 634-636.
31. Lommen A. MetAlign: interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data processing. Anal Chem., 81, 2009, 3079-3086.
32. Baran R, Kochi H, Saito N. MathDAMP: a package for differential analysis of metabolite profiles. BMC Bioinformatics,7, 2006.

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