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REVIEW ARTICLE

The Methods of Analysis in Forensics

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Abstract

Background: Forensic science in criminal investigations includes the examination and analysis of physical evidence.

Methodology: After studying numerous articles in this field; I found that this can include a number of sources of evidence, such as biological evidence (DNA or blood), trace evidence (fibres or hairs) and physical evidence (guns or fingerprints).

Conclusion: While the analysis of body evidence in an investigation is possible within forensic science, it is not the focus of the specialty. In contrast, forensic science relies on the inspection and analysis of physical evidence to help solve crimes and prosecute criminals.

Keywords: Toxicology, Forensics, Analytical Methods, Validation of Analytical Methods.

1. Introduction

Toxicology is an interdisciplinary branch (biology, chemistry, medicine) that deals with the study of the adverse effects caused by various chemical substances or physical agents on living organisms, or, in other words, the science of poisons and toxins. Adverse effects can occur at different levels of the body, for example only one organ, or only one tissue can be affected, but they can also occur at the level of the entire organism resulting in some situations even death [1].

Toxicology differs from pharmacology, although both disciplines seek to understand the properties of chemical substances and their action on living organisms. Pharmacology focuses mainly on the therapeutic effects of pharmaceutical substances and how they can be used most effectively for medical purposes. On the other hand, toxicology is related to the adverse effects that occur in living organisms when they come into contact with chemicals. More and more often new techniques appear with inestimable value in the examination of the facts, allowing the legal identification and quantification of a wide spectrum of matters. The extremely rapid evolution of science in recent years has determined the development of new, high-performance technologies that provide forensic experts with analytical capabilities unimaginable a few years ago. Therefore, the continuous improvement of the methods and technical equipment in the broad field of forensics, allows the specialists in this vast and complex field, the continuous processing and adaptation of new discoveries to judicial requirements. Some of the most important scientific discoveries that have been established as distinct branches within forensics [2].

For a long time, the development of DNA research was considered to be of only peripheral interest to forensics. In 1985, the communication presented by the English professor Alec J. Jeffreys, regarding the possibility of individual identification based on hypervariable repetitive areas of human DNA, revolutionized forensics.

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The research done in the human genome project, consisting in the identification of the DNA code of each living cell of an organism, demonstrated that this code is the support of heredity, in other words, the absolute unique imprint, of a genetic nature, of each individual [3].

DNA is a polymer, a very large molecule, formed by linking together a series of repeating units (nucleotides), numbering approx. Three billion. Nucleotides are divided into 4 types, designated, conventionally, by the letters ACGT (adenine, cytosine, guanine and tinine), grouped along a twisted band, whose structure has been designated with the name "double helix".

Those who managed to highlight the characteristics of the DNA molecule are the researchers James Watson and Francis Crick, in the year 1050, being rewarded, in 1962, with the Nobel Prize for discovering the structure of DNA [4].

From the very beginning, forensic scientists have asked the scientific world several questions, the answer to which is fundamental to the use of DNA analysis as evidence in criminal investigation: how large, physically speaking, must the biological sample be in order for laboratory analysis to highlight the DNA structure of an individual?; what is the maximum allowed age of such evidence, for it to be conclusive?; is there a specific area of the human body where the sample taken must be collected to be conclusive?; can human DNA be confused with the DNA of other living organisms or not?

To these questions, the researchers involved in the human genome project came up with a series of answers, clarifying each aspect separately. First of all, it has been scientifically proven that the size of the sample subjected to analysis for the determination of DNA can be reduced to the size of a molecule, DNA being present in absolutely all cells of a living organism, regardless of which part of the body they come from. Regarding the age of the biological samples, the dilemma was solved by research carried out on those taken from organisms whose age exceeds several thousand years [5].

A fascinating discovery led to the conclusion that not only the samples taken from the mummified, therefore preserved, organisms give results, but also the DNA analysis of some materials, used by the ancients in artistic or profitable activities, can lead to the identification of the DNA of some living organisms. DNA analyzes of some rock paintings discovered in the Lower Pecas region of Texas (2950 to 4200 years old) have led to the conclusion that the paints were composed of blood, urine, milk, eggs, vegetable juices and animal fats.

In forensics, the following is used most often, the DNA fingerprinting procedure, but also the usual methods of analytical chemistry and instrumental analysis; techniques characterized in the following paragraphs.

A fingerprint is a technique used by scientists to identify people, based on the characterization of DNA. DNA fingerprints are small variations in DNA that are generally different from one individual to another. DNA fingerprinting is different from whole genome sequencing. DNA fingerprinting is used, for example, in paternity tests and forensic investigations.

Although 99.9% of the DNA sequences are similar in all individuals, a significant portion of the DNA differs between individuals, except in monozygotic twins [6].

DNA fingerprinting uses repetitive sequences in DNA that vary widely from one individual to another, called "variable number tandem repeats," which are very similar between related individuals but very different between unrelated individuals.

2. DNA Fingerprinting Process

The DNA fingerprinting process begins with an individual's DNA sample (usually called a reference sample). DNA can be obtained from a sample of saliva, blood or semen, or from a personal item (toothbrush, razor blade) or biopsy tissue. Evidence obtained from blood relatives can be relevant in the process of identifying a person.

The reference sample is then analyzed to create the DNA fingerprint using a number of techniques, described below. The DNA fingerprint is compared to another sample to determine if there are genetic similarities [7].

3. Analytical Methods for Determining Professional Damages

Analytical methods are a set of principles and procedures that can allow the identification (qualitative determination) and possibly the dosage (quantitative determination) of a sample to be analyzed.

Qualitative analysis methods precede those of quantitative analysis, because the choice of quantitative determination methods depends on the nature of qualitatively identified substances and the

amount of sample. The Quantitative analysis methods include:

– gravimetry - chemical analysis is based on the exact weighing of a pure, solid substance from the analyte;

– volumetric analysis (volumetric analysis or titrimetry in which the concentration of the analyte in the sample is determined by precisely measuring the volume of reagent consumed - reagent in the form of a solution.

The volume measurement operation is called titration); physical-chemical methods:

– optical (UV, VIZ, IR molecular spectrometry, atomic absorption spectrometry, turbidimetry, nephelometry, X-ray spectrometry, fluorescence spectrometry, etc.);

– chromatographic (gas chromatographic, liquid chromatographic);

– electrochemical (polarography, conductometry, potentiometry, etc.);

– radiochemicals [8].

3.1 UV/VIS Spectrometry

Some substances (non-metals, metals, their compounds, organic substances) have the property of forming with specific reagents, under certain conditions, colored soluble compounds. The color intensity of the compound formed, called a colorimetric compound, is directly proportional to its concentration within certain limits.

The molecular spectrophotometric method is based on the determination (measurement, reading) of the absorbance or transmission of a colored solution of a specific concentration, at a specific wavelength, in the visible range, or of a colorless solution that absorbs in the ultraviolet. In the spectrum of electromagnetic radiation, the UV/Vis range is located between the X-ray region and the infrared region (10 nm – 800 nm). In industrial toxicology, the range 200-350 nm is used for UV and 350-800 nm for visible. Infrared spectrometry - the method is based on the interpretation of the IR spectrum of different types of chemical bonds specific to certain materials for the purpose of qualitative and/or quantitative identification of some chemical compounds. Infrared radiation (IR) represents that part of the electromagnetic spectrum, between the visible and microwave regions, which is characterized by wavelengths of the order of 10^{-5} m. To record the IR spectra used in determining the structure of organic and inorganic compounds, it is used only the mid-IR range, which contains wavelengths located in the region 2.5-25 μm (most often, the characterization is done using wave numbers in the range 400-4000 cm^{-1}). A conventional infrared spectrometer consists of a source that emits radiation in the IR range that is passed through the sample to be analyzed. The beam of radiation transmitted by the sample is passed through a monochromator to decompose the monochromatic radiation. The radiant energy is transformed into electrical pulses using a detector and which are amplified and recorded [9].

3.2 IR Spectrometer with Transform

Fourier has as its main component the Michelson type interferometer. The beam emitted by the source is passed through a collimator and then to a beam splitter. The splitter is made of a special material, so that half of the beam is sent to a movable mirror that moves. The movable mirror reflects the beam back to the splitter; it then reflects the beam through the sample to the detector. The other half of the beam is reflected by the splitter to a fixed mirror. This reflects the beam back to the splitter which reaches the detector through the sample. The two beams reflected by the two mirrors at the divider recombine, forming an interference figure. This figure is then transformed into a spectrum. The interferences that appear in IR spectroscopy are due to the fact that, in the case of a complex matrix, it is possible that one of the components of the matrix absorbs at the wavelength of the compound to be analyzed. This type of interference can be eliminated by a suitable choice of wavelength for the compound to be analyzed.

Atomic absorption spectrometry can be defined as a method for determining the concentration of an element in a sample by measuring the absorption of radiation in atomic vapors produced by the sample at a specific wavelength and characteristic of the element to be studied. Obtaining a population of free atoms is one of the main functions of atomic absorption devices. It can be done by analyzing the sample to be studied in the flame, electrothermally in the graphite furnace or with the help of the hydride generator. The flame appears as a result of a combustion process involving a fuel (acetylene) and an oxidant (air or nitrous oxide in the case of refractory elements). The flame is a source of free atoms and at the same time a space in which they are contained [10].

Although the flame analysis mode has notable advantages, the expansion of the use of the atomic

absorption spectrometry analysis method for different materials has also revealed some of its disadvantages, such as:

- the low efficiency of the atomization operation for some of the elements of the periodic system that can be determined by this technique;
- the use of a minimum sample volume of 4-5 ml which can only be decreased under special conditions;
- the existence of a large concentration of free radicals that can form in the flame and which then with the free atoms give stable compounds;
- the short time the atoms stay in the optical path.

To remove these deficiencies and especially when it is necessary to analyze the elements at the trace level, the graphite furnace is used. This system involves introducing the sample directly into a graphite tube using a micro-syringe. The volatilization of the sample is ensured by heating the tube to a high temperature (approx. 2500 ºC). The tube is protected by the circulation of a flow of argon. A water circuit ensures rapid cooling to room temperature after the measurements have been made. In the case of this method, the duration of a measurement is longer than in the flame analysis system due to the time allocated to each stage of heating the graphite furnace [11].

The hydride generator is used to determine the elements that can form hydrides (arsenic, selenium, antimony, bismuth, tellurium, tin, germanium, mercury) by the cold vapor method.

3.3 Chromatography - Method of Separation and Analysis

Chromatography - method of separation and analysis of chemical substances from a mixture that is based on the differential interaction of two or more separation compounds (solutes) with two chromatographic phases: the mobile phase and the stationary phase.

Gas chromatography (GC) - analyzes volatile organic compounds. The mobile phase is a gas and the stationary phase is usually a liquid on a solid support or absorbent solid.

Liquid chromatography (LC) - is used to separate analytes in solution. The mobile phase is a solvent and the stationary phase is a liquid on a solid support (eg "ion-exchange" resin) [12].

High-performance liquid chromatography (HPLC) - a variant of liquid chromatography that uses high pressure to increase the separation efficiency.

The main advantages of chromatography as a separation method are:

- allows the simultaneous separation, identification and quantitative dosing of the components of a mixture;
- it can be applied to a very large number of products, practically any organic compound can be separated by a chromatographic method;
- the sensitivity of chromatographic methods is extremely high, which means that they only require a very small amount of sample. At the same time, however, they can also be applied on a preparatory scale;
- the duration of the analysis is reduced, compared to other methods of analysis of complex mixtures.

The diagram that represents the result of the chromatographic analysis is called a chromatogram [13]. It is generally recorded as a function of detector signal strength versus time. The signal corresponding to each component that appears in the chromatogram is called a chromatographic peak, and the area of the peak is proportional to the concentration of the respective component [14].

X-ray diffractometry - X-rays (Roentgen rays), discovered in 1895 by the German physicist Roentgen, are electromagnetic waves with wavelengths between 10^{-5} Kx and 10^{2} Kx (1 Kx = 1.00202 A; 1A = 10^{-10} m).

The diffraction of X-rays by crystals is the basis of their use in studying the crystalline structure of materials. Since X-rays have wavelengths of the order of the distances between atoms, when they pass through the crystal a diffraction phenomenon occurs, instead of a single beam in the incident direction, obtaining several beams whose diffraction angles depend on the crystalline structure and the wavelength of the radiation. To record and measure the intensity of diffracted radiation beams, the method uses the ionization effect produced by these radiations, recorded by an instrument called a diffractometer. In toxicology, it is used to determine free crystalline silicon dioxide [15].

Indicative methods used in toxicology - colorimetric indicator tubes generally used to detect the presence of gases or vapors in the air of work areas. The principle of these tubes consists in passing a volume of air (with the help of a pump) through the tube equipped with a layer of reagent. When this reagent passes through the gas, it reacts with it, changing its color. Fast automatic

methods - for this purpose gas and/or volatile organic compound analyzers are used, which use for detection either electrochemical sensors - gas analyzers, or the photoionization properties of substances - volatile organic compound analyzers [16].

4. Validation of Analytical Methods

Analytical method validation – the process by which the laboratory establishes its performance characteristics and method limits and by which the factors that influence these characteristics and the degree of their influence are identified. Some of the parameters included in the validation methodology are:

- selectivity (specificity);
- precision;
- linearity;
- the field of work concentrations;
- accuracy or precision;
- detection and quantification limit.

Selectivity (specificity) – represents the extent to which the result of an analysis is influenced by the presence of other components.

Precision – the fit in a series of measurements obtained from several samples originating from the same parent sample.

Accuracy has several levels: - repeatability – the measure of the degree of dispersion in a confidence interval of the results obtained following the performed measurement; - intermediate precision – identical samples worked by the same method in the same laboratory with two or more different operating tools [17].

Linearity represents the ability of an analytical method to obtain results proportional to the concentration values of the analyte in the sample. The linear calibration function is given by the equation:

$$Y = a + bx$$

where, a - the intersection with the ordinate at the origin; b - the slope of the calibration line. The range of working concentrations – the range between the lower and upper concentration of the analyte in the sample to be analyzed for which it has been demonstrated that the procedure has a suitable level of precision, accuracy and linearity.

Accuracy or accuracy – the closeness between the real value and the value found in the sample to be analyzed. It is determined by the enrichment method:

$$R\% = [Cf - Ci]/Ca$$

where, R% - the recovery percentage of the analyte; Cf – the amount of analyte measured in the enriched sample; Ci - the amount of analyte measured in the unenriched sample; Ca – amount of analyte added. The recovery must have values between 85% and 105%. Detection limit – the lowest concentration that can be detected against the blank other than zero. Limit of quantification – the lowest concentration that can be determined with an acceptable level of repeatability and accuracy [18]. The limit of quantification must be lower than the lowest value in the field of working concentrations.

5. Conclusion

The term forensics refers to the scientific processes used in criminal investigations. When searching for forensic evidence, scientific knowledge must be used to solve or uncover evidence of a crime. Forensic investigation has several subcategories, including forensic medicine, forensic pathology, odontology, toxicology, forensic science, among many others. Regarding the existing strict delimitation between the DNA of living organisms, by species and subspecies, the researches of the last 25 years have decisively solved the problem. Today, the DNA structure of several species and subspecies is precisely known, but, above all, the year 2000 brought an absolute scientific novelty: the complete sequence of the human genome, the possibilities of making mistakes in identifying human DNA or in confusing it with the latter with the DNA of other living organisms, be it insects, animals or plants, being practically impossible. In this way, one of the main 11 conditions of criminalistics, namely the particularization and attribution of biological traces, regardless of their nature, taken from the scene of a crime, receives a complete answer - and complex at the same time - leading to solving some impossible cases to commit an error. The answers given by science to the legitimate questions of criminologists open perspectives beyond any limit for the interpretation of some traces taken from the scene of a crime for which, at the time of their taking, there were neither technical means nor sufficient scientific knowledge in order to interpret and complete the cases respectively. The fact that DNA analyzes can be performed on infinitesimal amounts of samples from living organisms, that their age does not constitute any impediment in their determination, as well as the creation of the complete map of the human genome, in the year 2000, which

excludes the possible confusions of Human DNA with DNAs dispersed in the genomes of lesser-known eukaryotic (non-human) species give DNA analyzes a unique, evidence-of-samples character. Under these circumstances, the discoveries about DNA are equivalent to the existence of a universal key for deciphering any code.

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