

Forensic Chemistry

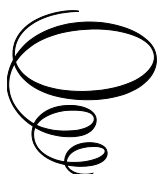
Forensic Chemistry:

Case Reports in Civil Applications

Edited by

Marcelo Firmino de Oliveira

**Cambridge
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Forensic Chemistry: Case Reports in Civil Applications

Edited by Marcelo Firmino de Oliveira

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PREFACE

This book is intended for chemistry students who plan to pursue a career in forensics, as well as for professionals already working in forensic chemistry. Most of the literature on forensic chemistry focuses primarily on criminal investigations. It is encouraging to see the growing number of books dedicated to the application of chemistry in crime scene analysis. However, this book aims to serve as a complementary resource, highlighting the potential for chemical knowledge to be applied in other areas of law, such as civil and labor cases.

Given the importance of chemistry in forensic science, this work provides an overview of chemical examinations conducted in civil contexts. Modern analytical techniques are introduced and discussed throughout the chapters, and each chapter includes at least one real case study to illustrate their forensic relevance.

The book is organized into ten chapters, contributed by outstanding collaborators, and covers topics such as general forensic chemistry, alcohol testing, adulteration of cosmetic products, chemical analysis in document examination, and more.

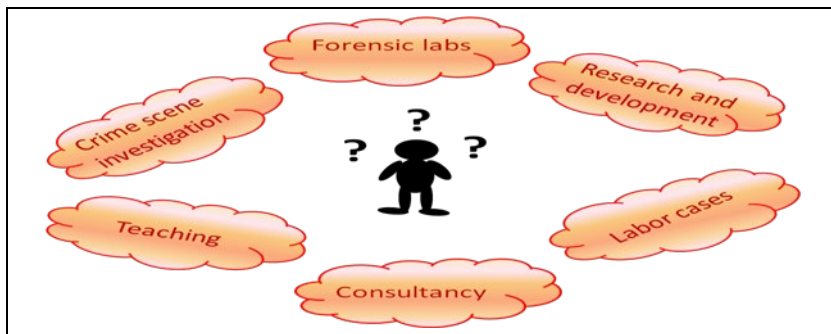
On behalf of all the authors, I hope this book inspires and supports readers in their pursuit of truth within the fascinating field of forensic chemistry.

—The Editor

CHAPTER 1

AREAS OF EXPERTISE IN FORENSIC CHEMISTRY

MARCELO FIRMINO DE OLIVEIRA



This chapter will address the ways in which chemistry professionals work in different forensic areas, such as civil, criminal, labor, and sports, among others, as well as the environments in which these professionals can work. A brief overview of the major initial areas of chemistry (analytical, biochemical, physical-chemical, inorganic, and organic) is discussed in this initial chapter, as well as the dynamics of the emergence of new interfaces between chemistry and other sciences, as well as the application of this knowledge in the legal area, culminating in the emergence of forensic chemistry. The work options include a wide range of activities, including crime scene analysis, laboratory analysis, teaching and research in forensic chemistry, as well as consulting activities. Each activity, in turn, demands constant updating of the chemistry professional, always keeping in mind the need to seek improvement. The interaction between chemistry and law constitutes the foundation of forensic chemistry and makes it possible to apply scientific knowledge in chemistry to the elucidation of legal cases whenever necessary.

Chemistry: a brief history

By dedicating oneself to the study of chemistry, students discover the immense amount of chemical reactions and processes that are part of our daily lives, whether involving substances of animal, plant, or mineral origin. Historically, all knowledge in chemistry has been grouped into five main areas of knowledge, according to the focus of each process: analytical chemistry, biochemistry, physical chemistry, inorganic chemistry and organic chemistry [1-4]. The number of distinct activities resulting from each of these major areas offers chemistry professionals a wide range of choices for their future professional career. Therefore, the activities carried out in each of these areas are briefly described below.

Fundamental areas of chemistry

Analytical Chemistry

Analytical chemistry is intended to study qualitative aspects (what is present in the composition of a chemical substance) and quantitative aspects (what is the content of each chemical substance in a given sample). Classical qualitative analyses include reactions performed on a semi-micro scale that involve color change, precipitation, gas evolution, among others. Classical quantitative analyses, in turn, require volumetric titrations, whether neutralization, precipitation, oxidation-reduction, or complexation. Currently, analytical chemistry has modern instrumental equipment that uses optical, electrical, thermal or separation methods, which provide faster, more accurate and sensitive information about the nature of a given sample.

Biochemistry

It involves the study of substances present in living beings, as well as the interactions of these substances with our organs, on a macroscopic or cellular scale. These substances, because they are generally synthesized in the organism itself, are called biomolecules, such as enzymes, proteins, amino acids, genetic material, among others. Although the composition of biomolecules is organic in nature, that is, formed by carbon chains (an area of activity of organic chemistry), they are studied in the area of biochemistry, since it is more prudent to separate the contents due to the gigantic volume of information, as well as the different applications of these substances in living organisms.

Inorganic Chemistry

This area of chemistry is dedicated to the study of substances formed by the other elements of the periodic table other than carbon, as well as their reactions. It can also include carbon compounds (carbonates, carbides, etc.), as long as they are not in the chain forms previously mentioned in organic chemistry. Among the matrices studied in this area, rocks, natural minerals, as well as the application of these substances in industrial processes can be mentioned.

Organic Chemistry

This area studies substances formed by carbon chains, as well as their respective reactions. Depending on the presence of other chemical elements in the carbon chain, as well as the existence of double and triple bonds between carbon atoms, different organic species can be obtained, such as alkanes, alkenes, alkynes, alcohols, ethers and esters, carboxylic acids, aldehydes, ketones, amines, amides and a huge combination of these for the production of various substances of human interest.

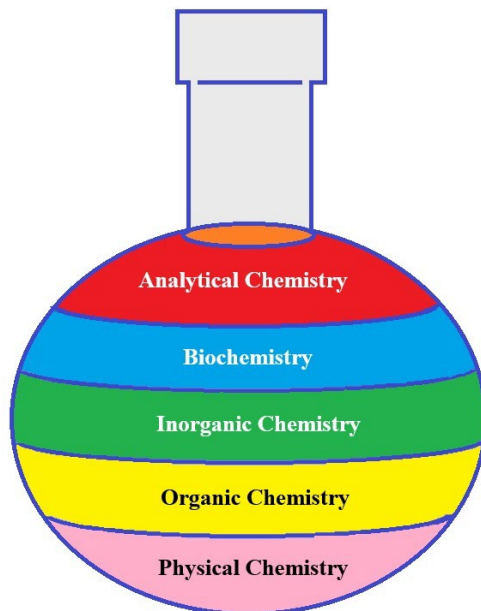


Figure 1.1: Five fundamental areas of chemistry.

Physical Chemistry

This area encompasses the energy aspects involved in chemical reactions, both on a macroscopic, molecular or atomic scale, using the principles of physics. Knowledge of the mass balance from analytical chemistry combined with the energy balance made possible by physical-chemical studies enables the application of chemical reactions with due safety for the operator, as well as on any desired production scale, in turn enabling better energy use and subsequent resource savings in a reaction process.

Evolution of science and the emergence of subareas

The demand for new processes, products and information arising from knowledge in chemistry, as well as the interaction of scientific knowledge in chemistry with new areas of knowledge, has enabled the emergence of several subareas of chemistry, which in turn constitute broad and complex sets of information, with the aim of developing and applying scientific and technological knowledge of chemistry to solve society's problems. Several subareas can be cited, according to their activity, such as environmental chemistry, computational chemistry, industrial chemistry, natural products chemistry, forensic chemistry, technological chemistry, polymer chemistry, petrochemistry, new materials chemistry, among others.

A survey conducted on the Web of Science scientific dissemination platform (www.webofscience.com) indicated that over the last 120 years, more than 11 million scientific articles containing the terms “chemistry” or “chemical” have been published. Figure 1.2 illustrates the 20 largest areas and subareas of chemistry cited in this research, as well as the number of scientific articles listed in each of them. In this survey, it is possible to see that the largest set of articles is located exactly in the topic of multidisciplinary chemistry, with almost 2.5 million articles.

Thus, we can observe a vast spectrum of professional activities available to chemistry professionals, with each line of work having specific attributions and requirements. In this context of multidisciplinaryity, this book discusses the intersection of knowledge in chemistry used in partnership with law to resolve legal cases. This is what Forensic Chemistry is all about.



Figure 1.2: 20 areas and subareas of chemistry with more publication of scientific articles in the last 120 years, being produced more than 11 million articles. Adapted from webofscience.com.

Forensic Chemistry

The term “forensic” comes from the Latin (forensis), which means “in open court, public”, for the forum. Thus, as in forensic chemistry, every area of knowledge that is preceded by the word “forensic” (forensic toxicology, forensic biology, forensic medicine, etc.) indicates the application of scientific knowledge from that area to help elucidate legal cases.

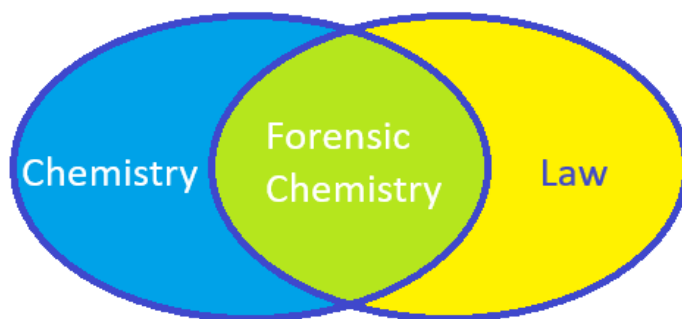


Figure 1.3: simplified definition of Forensic Chemistry.

According to another survey also conducted on the Web of Science (www.webofscience.com) scientific dissemination platform, this time using the keywords “chemistry” and “forensic”, it was found that over the last 120 years, more than 15,000 scientific articles have been published in this sub-area of chemistry. Figure 1.4, in turn, illustrates the 10 largest interactions between the areas and sub-areas of chemistry and forensic knowledge, cited in this research, as well as the number of scientific articles listed in each of them. In this survey, it is possible to see that the largest set of articles is in the area of analytical chemistry, with approximately 4,700 articles published.

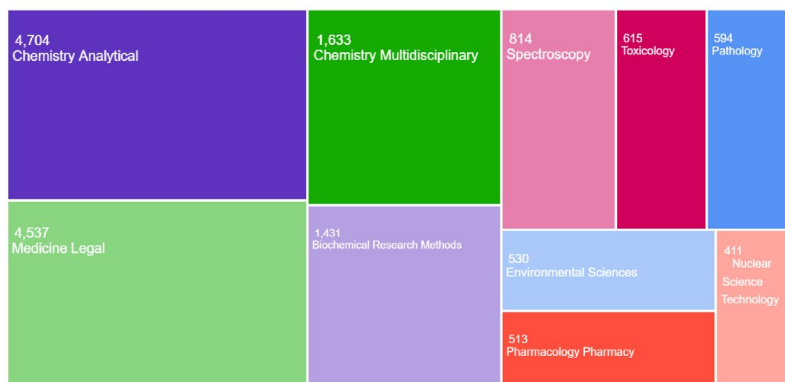


Figure 1.4: Interaction of the subareas of chemistry and law for the production of knowledge in forensic chemistry.

The application of chemical knowledge to solving cases of forensic interest has been widely carried out in different types of forums – criminal, civil, sports, etc. – but the dissemination of this area of knowledge has been primarily carried out in books on the criminal field. It is possible to contemplate works of both theoretical [5-13] and practical [14,15] aspects, which disclose chemical analysis techniques focused exclusively on crime scene analysis, leading the reader to the idea that it is only possible to work in forensic chemistry within the scope of criminalistics. The activities of crime scene analysis or analysis of parts and substances linked to a criminal act are exclusively police activities and must be carried out by civil servants who have passed a public examination in the police career. In this case, the forensic scientist is a civil police officer, subject to all the obligations inherent to a police career, such as carrying a firearm and traveling in police vehicles. Not every scientist aspires to work in a police career, for a variety of reasons. Thus, the purpose of this work is to

demystify this concept, exemplifying in this chapter other forms of action in forensic chemistry, enabling a wider range of choices for the chemistry professional, as well as prioritizing throughout this book several examples of action in the civil area.

Areas of actuation

The different forms of activity of the chemistry professional in the forensic area can be initially divided into two distinct initial sets:

- 1) regarding the type of court - criminal, civil, labor, sports, etc.,
- 2) regarding the form itself - criminal expert, researcher, professor, technical assistant and court expert.

Regarding the type of court

If the chemistry professional wants to work in laboratory analyses or expert external analyses, aimed at elucidating legal cases, he or she can work as a civil public employee in a police career (criminal cases) or as a freelance professional providing services (other cases).

Criminal court

In most countries, by law, all cases of crimes where material traces may have been produced at the scene are subject to official expert analysis, and such analyses cannot be substituted due to possible confessions by suspects.

In this context, it is possible to note the existence of official public agencies, both at the state and federal levels, dedicated to carrying out expert examinations at crime scenes. The chemistry professional interested in working for such agencies must have a degree in chemistry, as well as take a public exam to be admitted to the position. Upon passing the exam, the professional receives the title of Criminal Expert. This is a police position, in which the professional may carry out both analyses of external locations and laboratory analyses. Their work is exclusively in the criminal area.

INTERESTING FACT

In Brazil, for example, according to Article 158 of Decree-Law 3,689, of 10/03/1941:

Art. 158. When the offense leaves traces, a direct or indirect examination of the body of the crime will be indispensable, and the confession of the accused cannot replace it.

Sole paragraph. Priority will be given to the examination of the body of the crime when it involves a crime involving: (Included by Law No. 13,721, of 2018)

I - domestic and family violence against women; (Included by Law No. 13,721, of 2018)

II - violence against children, adolescents, the elderly or people with disabilities. (Included by Law No. 13,721, of 2018)

Civil court

Just as criminal cases constantly require expert analyses by professionals from other areas of knowledge – doctors, chemists, engineers, accountants, etc. – civil cases also require this. However, there are no public bodies linked to the judiciary or the police that have civil servants qualified to perform these examinations for the civil court. As already discussed in the previous topic, Criminal Experts work exclusively in criminal cases and should not meet demands from other courts. Thus, the judge responsible for a given civil case, upon determining the need for expert examinations to resolve a given legal demand, usually appoints a higher-level professional to perform such examinations.

This is a one-off contract, i.e., the professional designated to perform the tests – also called the Court Expert – performs the required tests and receives his/her fee after the expert report is delivered. Both laboratory tests and on-site tests in the civil area are very similar to those performed in the criminal area (the chromatographic chemical analysis equipment that performs, for example, the measurement of alcohol content in gasoline in a case of fuel adulteration, in a criminal area, is generally the same equipment that performs such a test in a case of accidental contamination, managed by the civil area). However, they are performed by professionals with different designations: one is a civil servant and a police officer; the other is a proven employee, not a police officer and hired on a one-off basis.

Labor court

All information described for working in the civil court applies to professionals with higher education who wish to work in cases originating from the labor court. In other words, the hiring of the Court Expert is specific to each case and does not involve an employment relationship. Labor cases in the chemistry area generally involve unhealthy conditions due to exposure to certain chemical products or hazardous conditions, for working with machines or chemical processes that involve greater risk.

Sports court

All information described for working in the civil court also applies to professionals with higher education who wish to work in cases arising from the sports court. In other words, the hiring of a Court Expert is specific to each case and does not involve an employment relationship. Cases in the sports area generally involve the analysis of chemical substances in biological fluids in anti-doping tests in sports competitions.

Regarding the form of actuation

Once a chemistry professional has chosen to work in the forensic field, he or she can perform expert examinations for the different types of forums discussed above, as well as teach training courses, and conduct scientific research to develop new analysis methods or improve current methodologies.

Criminal Expert

The role of a Criminal Expert consists of analyzing the evidence found at the crime scene, as well as the pieces collected at the scene. As previously mentioned, this is a public office and a police career, with all the duties that are inherent to it. The information collected and analyzed by the Criminal Expert is exclusively for the purposes of criminal court proceedings.

Functions of the Criminal Expert

In crime scene analysis, the functions of the Forensic Expert are:

- **To verify the offense:** Once the forensic team has been called to respond to a specific crime, it is first necessary to visualize the set of traces at the location in question to prove the materiality of the

crime. For example, armed robbery sites on public roads do not usually leave physical traces around them that could be used as the object of forensic study. Therefore, it is not possible to issue a report on this type of location and state that a crime occurred there. Unless the crime is photographed or filmed. In this case, image or video files constitute the traces to be forensically examined.

- ❖ **To classify the offense:** Once the offense has been confirmed, it is necessary to classify the location. There is a need to differentiate, for example, between locations of simple theft and qualified theft (when obstacles are broken to enter the premises), as well as to clarify whether a supposed suicide location is not in fact a disguised homicide location.
- ❖ **To collect evidences:** all material traces observed at the scene that may be linked to the crime must be collected and analyzed by the Criminal Expert, in order to determine the perpetrator of the crime, as well as the dynamics of the event. The traces may be visible to the naked eye, such as firearms, knives, spent cartridges, material damage to doors and windows, brake marks from traffic accidents, or they may be hidden, such as fingerprints, removed chassis numbers from vehicles and firearms, previously removed blood stains, etc. Hidden traces are also called latent traces and must be revealed through some physical or chemical process, so that they can be visualized by the Criminal Expert.
- ❖ **To perpetuate evidences:** Once traces that may be linked to criminal activity have been identified, there is a need to ensure proper preservation of the material evidence so that it can be used later throughout the entire judicial process. Fingerprints are examples. They are latent traces formed by water, fat, amino acids and mineral salts. Ultimately, they are fragile traces that can quickly disappear from the crime scene. After such traces have been revealed, either by colored powders or by vapors from revealing chemical substances, the prints can be properly photographed and digitized, becoming perpetual traces, free from modifications due to the action of time. Another example of perpetuating traces is the photographing and measuring of brake marks at the sites of traffic accidents. Despite being traces with good visual contrast, such marks tend to disappear from the surface of the roadbed after a few days, as a result of other vehicles passing over them. By measuring and photographing such traces, the Criminal Expert immortalizes the information in the judicial process, and can use it when necessary.

- ❖ **To legalize the evidences:** Finally, all information collected and analyzed by the Criminal Expert must be reported in the form of an official document called a technical expert report.

Actuations of the Criminal Expert

After entering the career of Criminal Expert, a professional with a degree in chemistry usually takes a training course in forensic examinations, and can learn and perform a wide range of forensic examinations for the exercise of his/her position. If he/she chooses to work in a chemistry laboratory, forensic analyses can be performed purely within the scope of chemical examinations. Additionally, the chemistry professional can also choose to work in crime scene attendance or examination of other pieces related to the case. Criminal Experts with a degree in chemistry can generally work in the following types of examinations:

- ❖ **crimes against property** (theft, robbery, damage, fraud and other frauds).
- ❖ **traffic accidents** (victims with bodily or fatal injuries).
- ❖ **crimes against the person** (homicide, suicide, bodily harm, etc.).
- ❖ **environmental crimes.**
- ❖ **various tampering** (vehicle chassis, gun serial numbers, documents, money bills, etc.).
- ❖ **ballistics** (various tests on weapons and projectiles).
- ❖ **laboratory tests** (chemistry, toxicology, physics and biochemistry).
- ❖ **DNA tests** (only for criminal purposes).

INTERESTING FACT

Forensic experts with a degree in chemistry CANNOT perform the following types of expert examinations:

- ❖ Engineering expertise.
- ❖ Computer expertise.
- ❖ Accounting expertise.

Court Expert

As previously mentioned, the Court Expert is an official expert in a given legal proceeding, whether in the civil, sports or labor areas. In other words, the professional with a higher education degree is appointed directly by the judge, according to his/her area of training. In this case, the professional with a degree in chemistry usually acts as a Court Expert in

legal proceedings where the demand for technical information is directly related to the area of chemistry.

Functions of the Court Expert

As the official expert in a given court case, the functions of the Court Expert are the same as those of a Criminal Expert, that is, to confirm the type of examination, qualify the examination, collect the evidence, perpetuate the evidence and finally, legalize the evidence in the form of a technical expert report.

Actuations of the Court Expert

Considering the activities of the Court Expert in the area of chemistry, he/she generally works on the following types of tests:

- ❖ **blood alcohol tests** (for civil or compensatory purposes).
- ❖ **quality control of chemical products** (adulterations in cosmetics, insecticides, veterinary products and others).
- ❖ **objections of food products** (presence of foreign bodies in food and beverages).
- ❖ **instrumental analyses of documents** (spectrometric, microscopic, chromatographic and electrochemical analyses of chemical substances present in printing inks and signatures).
- ❖ **analysis of physical-chemical properties of commercial products** (checking the granulometry of solids for the purposes of correct taxation).
- ❖ **recalculation of taxes** (checking the correct classification of chemical products according to the Harmonized System of Designation and Coding of Goods - HS).
- ❖ **laboratory tests** (Chemistry, Toxicology, Physics and Biochemistry for civil purposes).

Technical Assistant

The Technical Assistant is a graduated professional with previous experience in forensic analysis (usually someone who has already worked as a Criminal Expert or Court Expert) who is hired by one of the parties involved in a legal proceeding in any area (criminal, civil, labor or sports). Our society commonly knows that, when participating in any legal proceeding, people have the right to a lawyer for their defense. However, they are generally unaware that they can also hire a professional with a

higher education level and experience in the subject matter of the proceeding, to assist in their defense. For example, in a traffic accident proceeding, if one of the parties feels harmed by the conclusions of the Criminal Expert, they can hire a private expert – a Technical Assistant with previous experience in this type of expert service – to issue an opinion on possible contradictory information in the official report.

Unlike the Criminal Expert and the Court Expert, the Technical Assistant does not perform the first expert examinations on site or on the examination documents involved in the legal proceedings. He begins his work after the legal proceedings have begun, often having to resort to images of photographs of reports and other official documents. His expert examinations are often carried out indirectly, that is, they basically consist of an analysis of the official report issued by the official expert in the case.

Although this professional almost always acts as a critic of the official report, it is important to know that every person has a full right to defense in a legal proceeding and that, if there are really untruths or inconsistencies in the official documents, such errors must be reported in the process. Ultimately, the more scientific discussion there is about a given topic, the closer the forensic environment will be to the truth.

Professor

A chemistry student who is enthusiastic about the subject of “forensic chemistry” can direct his or her efforts towards completing postgraduate studies (master’s and doctorate) with projects focused on this topic. Upon becoming a university professor, he or she can teach subjects related to the forensic area. It is clear that this professional may not have performed any type of expert examination in legal proceedings, but still acquire a theoretical/practical basis that qualifies him or her to work in teaching forensic chemistry. Fortunately, it is currently possible to observe a vast specialized literature both in forensic chemistry and in forensic sciences in general [5-15], where the professional dedicated to forensic chemistry can consolidate his or her technical-scientific training.

Researcher

Forensic chemistry research activities are generally associated with universities. When hired full-time for teaching and research, the University Professor also acts as a Researcher, and may conduct research into new methods of forensic analysis or the development of new substances to be

used in site examinations or trace detection. In practice, the Research Professor acts as a supervisor for undergraduate and graduate students, who effectively carry out the research activities proposed by the supervisor.

Motivating factors for this work

An analysis of the teaching material currently available for the study of forensic chemistry, whether in the form of books or scientific articles, indicates that it is possible to verify the state of the art in forensic analysis. Several works dedicated to this subject describe the most modern techniques of chemical analysis, as well as applications in analysis of examination sites and parts. However, it was possible to verify the presence of certain gaps in the set of information on the application of forensic chemistry, which are discussed below.

Demystifying the concept of forensic chemistry

Although the collection of teaching materials available to date is of excellent quality, it is still possible to see that most of this material associates forensic chemistry exclusively with crime scene analysis. Knowledge of chemistry really helps in a powerful way in the expert analysis of crime scenes and the pieces involved in the crime: fingerprint searches, blood detection, analysis of drugs, explosives, fuels, blood alcohol tests, etc. However, practically all the same laboratory chemical tests can be applied in expert analyses in other forums, such as civil, sports and labor. If the results of a laboratory chemical analysis are to be used in a legal proceeding, then such tests are also forensic.

Lack of didactic materials

Consequently, although there is a wide range of books devoted to the subject of forensic chemistry for criminal purposes, it has not been possible to find to date a work devoted to forensic chemical examinations in the civil area. This book therefore aims to report some case studies involving forensic chemistry in the civil area, demonstrating the applicability of knowledge in chemistry in this judicial sphere.

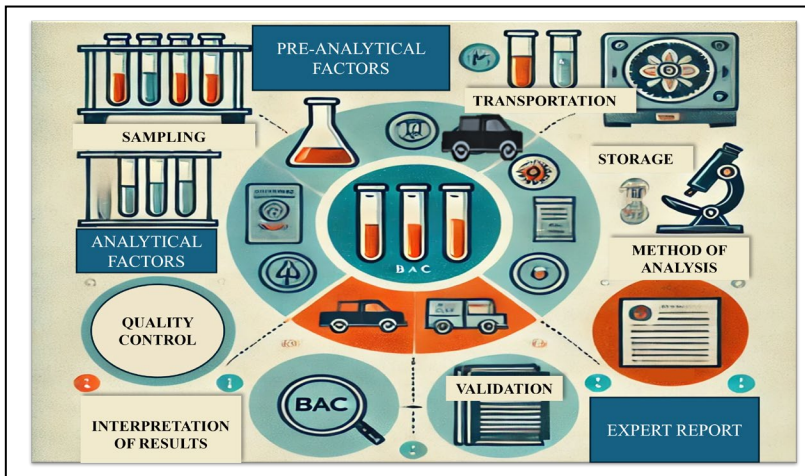
Expanding the range of professional activities of the chemist

In addition to all the tasks that a forensic chemistry professional can perform in the criminal field, which are constantly described in specialized literature in this area, it is possible to see that the aforementioned professional can also act in ways that complement those of the Criminal Expert. The chemistry professional can be a Court Expert, a Technical Assistant, a Teacher or a Researcher, if he or she so wishes. The choice should be guided by your personal inspiration.

CHAPTER 2

BLOOD ALCOHOL CONCENTRATION IN POST-MORTEM SAMPLES

MARAINÉ CATARINA TADINI
AND MARCELO FIRMINO DE OLIVEIRA



The determination of blood alcohol concentration (BAC) in post-mortem samples from traffic accident victims is a critical toxicological analysis in forensic investigations. Due to the high demand for such evaluations, various pre-analytical aspects and analytical considerations must be meticulously addressed, particularly within the forensic context. This chapter aims to delineate the essential elements associated with the chain of custody for blood samples obtained from cadavers, as well as the most important analytical factors required to ensure the reliability and validity of results from BAC. Finally, a comprehensive list of criteria for preparing robust and defensible forensic reports will be presented.

About the court case

The request originated from a traffic accident in which the vehicle's driver was the fatal victim. A police report was filed, followed by routine post-mortem examinations, during which a blood sample was collected from the victim's body. The blood alcohol concentration (BAC) analysis was performed 16 days after the death, with the ethanol results being reported 30 days post-mortem. The official forensic report indicated a BAC of 0.61 g L^{-1} , slightly exceeding the legal limit established by Brazilian law for civil and criminal infractions, which was 0.60 g L^{-1} in the existing legislation at time (Brazil, 2013; Brazil, 2008).

Considering the positive result obtained for a BAC, the victim's family decided to appeal the official results by consulting a second opinion from a forensic chemist.

A LITTLE BIT OF HISTORY

In Brazil, the Law No. 11,705, known as the "Dry Law," was enacted in 2008 (Brazil, 2008). This law amended the Brazilian Traffic Code, categorizing driving a vehicle after consuming alcohol as a very serious offense. It introduced administrative penalties such as the suspension of the driver's license for a specified period, fines, and/or vehicle impoundment (Brazil, 2008). Additionally, the law allowed for criminal penalties if the blood alcohol concentration (BAC) exceeded 0.6 g L^{-1} , including fines, suspension or revocation of the driver's license, and even imprisonment for a pre-determined period in a correctional facility (Brazil, 2008; Andreuccetti *et al.*, 2011). With over a decade in effect, it is evident that there has been a significant reduction in traffic accidents and fatalities (Andreuccetti *et al.*, 2011). Other countries have also implemented laws addressing "safe" alcohol consumption or prohibiting ethanol consumption by drivers, with varying BAC thresholds (Jones and Tilson, 2023).

What problem is presented

A forensic chemist analyst possesses significant expertise in blood alcohol concentration (BAC) analysis. This professional can contribute across multiple stages, from ensuring the integrity of samples throughout the chain of custody to performing the BAC analyses and preparing technical documents, such as the official forensic report or expert opinion.

The chain of custody is a critical aspect of BAC analysis, as it guarantees the integrity of collected samples. A forensic chemist has technical knowledge to guard and evaluate each stage of chain of custody process, ensuring that proper procedures for storage and transport are rigorously followed. This diligence safeguards the validity and reliability of the results obtained.

In the execution of BAC analysis, the technical and scientific knowledge of chemists is indispensable. And a forensic chemist can accurately and precisely determine the concentration of ethanol in a sample by advanced techniques such as gas chromatography.

The preparation of technical reports is another area where the expertise of the forensic chemist stands out. These documents assist judicial decision-making.

CURIOSITY

Currently, ethanol consumption is strongly associated with traffic accident fatalities in several countries. For instance, in 2022, 32% of traffic fatalities in the United States were linked to drivers under the influence of ethanol—BAC ≥ 0.08 g dL⁻¹ (U.S. Department of Transportation and NHTSA, 2024). Therefore, we consider that ethanol consumption by vehicle drivers should be discouraged.

Form of action

In this case study, the experts acted as technical-forensic consultants. They conducted a thorough evaluation of the procedures followed in maintaining the chain of custody for the blood sample collected from the deceased. Additionally, they critically assessed the blood alcohol concentration (BAC) analysis performed by the police laboratory, verifying the accuracy and precision of the employed methods, as well as interpreting the results presented in the official report.

Description of the methodology

The methodology used in the preparation of the expert opinion involved an extensive literature review to establish a solid theoretical foundation. This review also facilitated the detailed description of the key aspects surrounding the chain of custody for blood samples collected post-mortem and the main challenges encountered in ethanol quantification analyses.

Pre-analytical factors in ethanol quantification of post-mortem blood samples: general aspects

The detection and quantification of ethanol in post-mortem blood samples is a critical toxicological test in forensic investigations, primarily aimed at determining whether the victim was under the influence of alcohol at the time of death (Ialongo, 2024). Ethanol is a hydrophilic molecule that is rapidly absorbed into body tissues and fluids through simple diffusion (Marti *et al.*, 2021; Savini *et al.*, 2020). Consequently, biological compartments with high water content, such as blood, urine, and vitreous humor, tend to exhibit higher ethanol concentrations, making these the primary matrices employed in forensic analyses (Savini *et al.*, 2020).

Pre-analytical factors encompass all aspects related to the biological matrix prior to sample analysis (Olds and Jones, 2024), as illustrated in Figure 2.1.

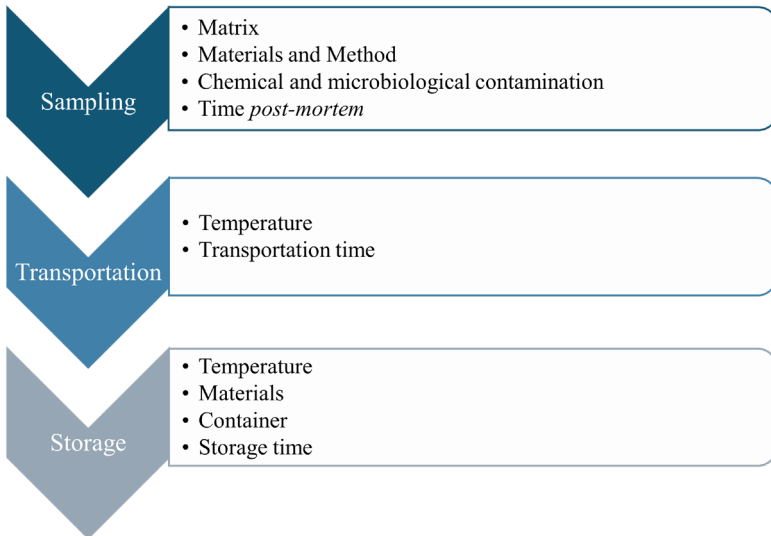


Figure 2.1. Pre-analytical factors associated with ethanol analysis in post-mortem biological samples

Pre-analytical factors primarily encompass the conditions of sample collection, transportation, and storage. These factors must be meticulously detailed in the necropsy report and reinforced in the analytical report. Key considerations include the post-mortem phase, the nature of the biological

matrix sampled, the sampling methodology and the materials, the environmental conditions during sample transportation, the temperature of sample storage, as well as the time of sample storage, and others (Ialongo, 2024; Kugelberg and Jones, 2007; Maletin *et al.*, 2024).

Numerous pre-analytical factors are associated with the procedures employed by the forensic experts, enabling the implementation of improved protocols to minimize potential errors (bias). This ensures greater accuracy in results and enhances the reliability of the forensic report (Ialongo, 2024). In the following sections, we will discuss examples of relevant pre-analytical factors that are essential for ensuring the reliability of analytical results.

Sampling of post-mortem blood

Firstly, it is essential to consider the potential for chemical contamination by ethanol, particularly in cases of cross-contamination arising from the use of alcohol during resuscitation procedures or in the antiseptics of the skin prior to the introduction of surgical instruments (Ialongo, 2024). Therefore, the use of non-alcoholic antiseptics for blood sampling is strongly recommended in these scenarios (Ialongo, 2024; Olds, Kosecki and Jones, 2023).

Another possible source of chemical contamination is the post-mortem transfer of ethanol from the stomach to the bloodstream via passive diffusion (Sastre *et al.*, 2013). During the necropsy examination, it is advisable to prioritize phlebotomy of peripheral veins, such as the femoral or iliac veins, and to perform this procedure before evisceration or embalming to avoid the redistribution or endogenous production of ethanol (Dinis-Oliveira, Vieira and Magalhães, 2016; Olds and Jones, 2024; Sastre *et al.*, 2013).

Additionally, microbiological contamination can occur during sampling, potentially introducing anaerobic bacteria into the collection tubes (Ialongo, 2024). Consequently, forensic pathologists should be vigilant in executing procedures that minimize the risk of chemical or biological contamination.

Furthermore, depending on the post-mortem phase, ethanol may be produced post-mortem due to the fermentation of glucose in the blood by microorganisms such as *Candida albicans*, *Clostridium sp.*, and *Escherichia coli* (Dinis-Oliveira, 2024; Olds and Jones, 2024). Thus, the forensic