# TOXICOLOGICAL ANALYSIS OF HAIR IN FORENSIC TOXICOLOGY: APPLICATIONS, LIMITATIONS, AND CLINICAL AND EXPERT PERSPECTIVES


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**Abstract:** Hair toxicological analysis has become an important tool in clinical and forensic toxicology due to its ability to detect long-term exposure to drugs of abuse, medications, and other chemical substances. Unlike conventional biological matrices such as blood, urine, and saliva, hair provides a wide detection window, allowing the reconstruction of an individual's history of substance use over weeks or months. This study aimed to analyze the applications and limitations of hair toxicological analysis in clinical and forensic investigations, highlighting its relevance for detecting psychoactive substance use, the analytical methods employed, and the challenges associated with result interpretation. This is a qualitative, descriptive bibliographic review based on the analysis of scientific articles, books, international guidelines, and technical documents retrieved from national and international databases. The findings demonstrated that techniques such as Gas Chromatography–Mass Spectrometry (GC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) provide high sensitivity and specificity, contributing to the reliability of toxicological examinations. However, factors such as external contamination, hair pigmentation, cosmetic treatments, hair growth rate, and the lack of universal standardization in result interpretation remain significant limitations to their application. It is concluded that hair toxicological analysis is a valuable scientific tool for clinical and forensic investigations, provided that results are interpreted comprehensively by considering laboratory, clinical, and forensic aspects, together with validated analytical protocols and internationally recognized technical guidelines.

**Keywords:** Forensic investigation; Forensic toxicology; Hair toxicological analysis; LC-MS/MS; Forensic sciences.

## INTRODUCTION

Forensic toxicology is a multidisciplinary area of Health Sciences that plays a fundamental role in the investigation of events related to the use of psychoactive substances, medications, poisons and other chemical agents capable of causing physiological changes or contributing to the occurrence of crimes, accidents and deaths.

Its work integrates knowledge from toxicology, pharmacology, analytical chemistry, forensic medicine and molecular biology, allowing the identification, quantification and interpretation of the presence of substances in different biological matrices.

In this context, toxicological tests represent important tools to support clinical, forensic and judicial decisions, providing scientific evidence that helps in the reconstruction of facts and in the determination of exposure to chemical compounds (Levine, 2020).

Traditionally, blood, urine and saliva samples are widely used in toxicological investigation due to the ease of collection and the ability to detect recent drug and medication use. However, these matrices have limitations related to the short detection period of the substances, making it difficult to identify exposures that occurred weeks or months before collection.

In view of this limitation, toxicological analysis in hair has been gaining prominence in clinical and forensic toxicology because it allows a significantly wider detection window, allowing the evaluation of patterns of chronic or repetitive consumption of drugs of abuse, medications, and other chemical substances over time (Cooper; Kronstrand; Kintz, 2019).

Hair has biological characteristics that make it a differentiated matrix for retrospective investigations. During its growth, substances present in the bloodstream can be incorporated into the hair structure, remaining stable for months or even years, depending on the length of the sample analyzed.

Considering that the average growth of human hair is approximately one centimeter per month, the segmentation of the strands makes it possible to establish an approximate chronology of exposure to substances, a characteristic that is especially relevant in criminal investigations, labor forensics, therapeutic monitoring, abstinence programs, child custody processes, monitoring of drug users and evaluations related to professional aptitude (Kintz, 2018).

The technological advances observed in recent decades have contributed significantly to the improvement of capillary toxicological analysis. Highly sensitive and specific instrumental techniques, such as gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), allow the identification of extremely low concentrations of different chemical compounds with high analytical precision.

These methods have significantly reduced the occurrence of false-positive and false-negative results, increasing the reliability of the tests and strengthening their use as scientific evidence in court proceedings and forensic investigations (UNODC, 2023).

Despite the numerous advantages, the interpretation of the results obtained through hair analysis remains one of the greatest challenges of contemporary forensic toxicology. Several factors can interfere with the incorporation and permanence of substances in the hair, including individual characteristics, hair color and type, cosmetic treatments, frequency of exposure, metabolism, route of drug administration and, especially, the possibility of external contamination.

The environmental deposition of drugs by smoke, dust, or physical contact can lead to results that require careful laboratory evaluation, making it essential to adopt strict protocols for decontaminating samples before performing analyses (Society of Hair Testing, 2023).

In addition to the limitations related to external contamination, there are still challenges regarding the international standardization of reference values, analytical cutoff points, and interpretative criteria used by different laboratories. These differences can directly influence the clinical and expert interpretation of the results, especially in situations involving criminal liability, investigation of suspicious deaths, occupational monitoring, or assessment of abstinence in chemical dependency treatment programs.

Thus, the interpretation of the tests must consider not only the laboratory results, but also the clinical context, the individual's history, the sample's chain of custody, and other available evidence.

In view of the growing use of toxicological analysis in hair in clinical and forensic practice, it is necessary to critically discuss its potentialities, limitations and applications.

Thus, the present study aims to analyze, through a review of the scientific literature, the main applications of toxicological analysis in hair in clinical and forensic investigation, addressing its detection window, the analytical methods employed, especially LC-MS/MS and GC-MS, the factors that can interfere in the results, including external contamination, as well as the challenges related to the interpretation of toxicological tests.

It is hoped that this review will contribute to broadening the understanding of this important diagnostic and forensic tool, highlighting its relevance for the Health Sciences and for the production of reliable scientific evidence in the field of forensic toxicology.

## OBJECTIVES

### General Objective

To analyze the applications and limitations of toxicological analysis in hair in clinical and forensic investigation, highlighting its importance as a tool for detecting the use of psychoactive substances, the analytical methods employed and the challenges related to the interpretation of the results.

### Specific Objectives

Describe the principles of toxicological analysis in hair and its use in clinical and forensic toxicology.

Discuss the detection window provided by capillary analysis compared to other biological matrices.

To present the main analytical techniques used in the identification of substances, with emphasis on gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

To analyze the limitations of toxicological analysis in hair, especially the risks of external contamination and the factors that interfere in the incorporation of substances into the hair.

Discuss the challenges related to the interpretation of toxicological results and their application in clinical practice and forensic investigation.

## MATERIALS AND METHODS

The present study is characterized as a bibliographic research, with a qualitative approach and descriptive nature, developed with the objective of analyzing the applications and limitations of toxicological analysis in hair in clinical and forensic investigation.

Bibliographic research allows us to gather, organize and interpret scientific knowledge already published, providing a broad understanding of the current state of the investigated theme. According to Gil (2019), this type of research is based on the systematic analysis of materials already prepared, enabling the construction of critical discussions based on scientific evidence.

To carry out the research, national and international databases recognized in the area of Health Sciences were consulted, including Scientific Electronic Library Online (SciELO), PubMed/MEDLINE, Virtual Health Library (VHL), Scopus and Google Scholar. Technical documents and guidelines published by reference organizations, such as the United Nations Office on Drugs and Crime (UNODC), the Society of Hair Testing (SoHT) and the European Workplace Drug Testing Society (EWDTS) were also used, considering their relevance for the standardization of toxicological tests and for the interpretation of results in different clinical and expert contexts.

The search strategy was carried out by combining descriptors in Portuguese and English, using the Boolean operators AND and OR to expand the retrieval of pertinent studies. Among the main descriptors used are: "forensic toxicology", "toxicological analysis in hair", "hair analysis", "hair testing", "forensic toxicology", "LC-MS/MS", "GC-MS", "detection window", "external contamination", "toxicological interpretation" and "biological samples".

The use of these terms made it possible to identify studies related to the clinical and forensic applications of hair analysis, the analytical methods employed and the factors that interfere in the interpretation of toxicological tests.

Inclusion and exclusion criteria were established to ensure the quality and timeliness of the information analyzed. Scientific articles published preferably between 2018 and 2025, literature reviews, experimental studies, technical guidelines, and official documents related to forensic toxicology, toxicological examinations of hair, and instrumental chromatography techniques coupled to mass spectrometry were included.

Classics widely recognized in the literature were also considered when indispensable for the conceptual foundation of the theme. Duplicate studies, publications without full text available, studies that did not directly address toxicological analysis in hair, and documents whose methodology had limitations that compromised the reliability of the results were excluded.

After selecting the studies, an exploratory reading was carried out, followed by an analytical and interpretative reading of the included publications. The extracted data were organized in a thematic way, covering aspects related to the applications of toxicological analysis in hair, the detection window of the substances, the analytical techniques used, especially gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), as well as the limitations arising from external contamination,  the biological factors that influence the incorporation of substances into the threads and the challenges involved in the interpretation of laboratory results.

The data analysis was conducted in a descriptive and critical manner, seeking to compare the evidence available in the scientific literature and identify consensuses, divergences and gaps related to the use of toxicological analysis in hair in clinical and forensic investigation.

## RESULTS AND DISCUSSION

### Toxicological analysis in hair and its use in clinical and forensic toxicology

Hair toxicology analysis has established itself as an important tool in clinical and forensic toxicology because it enables the identification of prolonged exposure to drugs of abuse, medications, alcohol and other chemical substances. Unlike blood, urine, and saliva, this biological matrix allows you to evaluate the history of consumption for weeks or months, making it especially useful in forensic investigations, therapeutic monitoring, and clinical follow-up.

According to Kintz (2018), the main advantage of hair analysis is the wide detection window, which makes it possible to reconstruct the temporal profile of exposure to substances and identify chronic patterns of consumption or withdrawal.

The incorporation of substances into the hair occurs mainly during the anagen phase of hair growth, when compounds present in the bloodstream reach the hair follicle and remain retained in the keratin structure. Cooper, Kronstrand and Kintz (2019) explain that this process occurs predominantly by blood diffusion, although mechanisms related to sweat and sebum can also contribute to the deposition of analytes.

For Levine (2020), this characteristic differentiates hair from other biological matrices, as it allows obtaining retrospective information that would be difficult to identify by blood or urine tests performed days after exposure.

In clinical practice, capillary analysis has been widely used in the follow-up of patients undergoing treatment for chemical dependency and in the monitoring of treatment adherence. Kintz (2023) highlights that the technique makes it possible to identify relapses and monitor the evolution of the treatment through segmental analysis of the hair. Musshoff and Madea (2021) add that this approach allows for the estimation of different periods of consumption, offering important chronological information for clinical evaluation and therapeutic planning.

In the field of forensic toxicology, hair analysis has high probative value, being used in investigations of suspicious deaths, traffic accidents, domestic violence, sexual crimes, legal disputes related to child custody and monitoring of individuals subjected to legal measures.

According to the United Nations Office on Drugs and Crime (UNODC, 2023), the chemical stability of the sample, the ease of storage, and the lower susceptibility to adulteration make this matrix especially useful in the production of scientific evidence. Similarly, the European Workplace Drug Testing Society (EWDTS, 2022) recognizes its importance in monitoring professionals who perform risky activities, as long as the results are interpreted in conjunction with clinical and occupational assessment.

Despite its numerous advantages, the literature highlights that toxicological analysis in hair has limitations that require careful interpretation. Pragst and Balikova (2006) demonstrated that factors such as hair pigmentation, cosmetic treatments and environmental contamination can influence the incorporation of substances into the hair.

Corroborating this perspective, Kintz (2023) states that the mere detection of a drug in the hair does not confirm, in isolation, the frequency or intensity of consumption, and it is essential to consider the clinical context, laboratory data, and other evidence of the investigation.

Thus, there is a consensus among the main authors regarding the relevance of toxicological analysis in hair for clinical and forensic toxicology. As highlighted by Cooper, Kronstrand and Kintz (2019) and Levine (2020), the reliability of this methodology depends on the use of validated analytical techniques, the adoption of standardized protocols and the specialized interpretation of the results.

Thus, when used judiciously, capillary analysis is a tool of high diagnostic and expert value, contributing significantly to the production of scientific evidence in the Health Sciences and in forensic investigation.

**Detection window of capillary analysis and comparison with other biological matrices**

The choice of the biological matrix is an essential step in toxicological investigation, as each type of sample has specific characteristics related to the period of detection of the substances, ease of collection, and clinical or forensic applicability. Among the most used matrices are blood, urine, saliva and hair, the latter being recognized for its wide detection window, capable of identifying the consumption of drugs and medications weeks or months after exposure (Levine, 2020).

The detection window corresponds to the period in which a substance remains detectable in a given biological matrix. According to Kintz (2018), this interval depends on the pharmacokinetic characteristics of the drug, the frequency of use, the dose administered, the individual metabolism, and the type of sample analyzed. Thus, the selection of the matrix should consider the purpose of the examination and the time elapsed since exposure.

Blood is the most suitable matrix to evaluate acute intoxication and the immediate effects of psychoactive substances. Musshoff and Madea (2021) state that blood concentrations reflect the individual's state at the time of collection, and are widely used in traffic accidents and medico-legal investigations. However, Levine (2020) points out that its short detection window limits the identification of exposures that occurred several days ago.

Urine remains one of the most used matrices in screening and monitoring programs for drug use. According to the European Workplace Drug Testing Society (EWDTS, 2022), it allows metabolites to be identified for a period that generally varies between one and seven days. However, Cooper, Kronstrand and Kintz (2019) point out that this test only confirms previous exposure, without indicating the exact moment of consumption or the degree of impairment of the individual.

Saliva, in turn, has a simple and non-invasive collection, and is indicated to detect very recent consumption. According to the United Nations Office on Drugs and Crime (UNODC, 2023), this matrix mainly reflects the free fraction of substances present in the bloodstream, which is why it is often used in traffic inspections. However, its reduced detection window restricts its application in retrospective investigations.

In contrast, toxicological analysis on hair allows you to reconstruct the history of consumption over long periods. Kintz (2023) explains that, considering the average growth of approximately one centimeter per month, a six-centimeter sample can represent about six months of exposure. In addition, Cooper, Kronstrand and Kintz (2019) point out that the segmental analysis of the strands makes it possible to identify different periods of consumption, making this technique especially useful in therapeutic follow-up and forensic investigations.

Despite these advantages, hair analysis has limitations. Levine (2020) observes that there is an interval of approximately seven to fourteen days between the consumption of the substance and its incorporation into the visible segment of the hair, making it impossible to detect very recent exposures.

In addition, factors such as hair pigmentation, hair growth speed, and cosmetic treatments can interfere with the concentrations detected. Pragst and Balikova (2006) demonstrated that more pigmented hair tends to incorporate greater amounts of certain drugs, evidencing the need for careful interpretation of the results.

According to the Society of Hair Testing (SoHT, 2023), no biological matrix should be considered superior to the others, as each one meets specific objectives. While blood and saliva are more suitable for identifying recent consumption, urine is widely used in monitoring programs, and hair stands out for its ability to document prolonged exposure. Thus, the scientific literature demonstrates that capillary analysis does not replace the other matrices, but complements them, increasing diagnostic accuracy and strengthening clinical and forensic investigation when the results are interpreted in an integrated manner.

## Analytical methods used: GC-MS and LC-MS/MS in toxicological analysis of hair

Advances in analytical techniques have significantly strengthened forensic toxicology, allowing the identification of chemical substances in increasingly smaller concentrations and with high reliability. In toxicological analysis of hair, instrumental methods are fundamental due to the low concentration of drugs incorporated into the capillary matrix. Among the main techniques used, gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) stand out, considered reference methods for analytical confirmation in clinical and forensic laboratories (UNODC, 2023).

Before instrumental analysis, the sample undergoes rigorous laboratory preparation. Cooper, Kronstrand and Kintz (2019) describe that this process involves standardized collection, decontamination, drying, fragmentation and extraction of the analytes present in the hair. According to the authors, these steps are essential to ensure the reliability of the results, since environmental residues can remain adhered to the threads and compromise toxicological interpretation.

In this context, the Society of Hair Testing (SoHT, 2023) recommends specific washing protocols to remove external contaminants without significantly altering the substances incorporated into the interior of the hair fiber.

After decontamination, the substances present in the keratin are extracted. Levine (2020) points out that different digestion and extraction methods can be used, depending on the physicochemical properties of the analyte and the analytical technique used. Musshoff and Madea (2021) add that efficient extraction increases the recovery of compounds and improves the sensitivity of the exams, making the results more reliable for clinical and forensic applications.

GC-MS remains one of the most consolidated techniques in forensic toxicology. Levine (2020) explains that this method separates volatile compounds by gas chromatography and performs their molecular identification by mass spectrometry, presenting high specificity and reducing the occurrence of false-positive results. However, Cooper, Kronstrand and Kintz (2019) observe that some substances require chemical derivatization before analysis, making the procedure more complex and limiting its use to certain thermolabile compounds.

On the other hand, LC-MS/MS has expanded its use due to its ability to analyze polar and thermally unstable molecules without the need for derivatization. According to Kintz (2023), this technique has high sensitivity, speed, and versatility, allowing the simultaneous detection of different drugs and their metabolites in a single analysis. Levine (2020) reinforces that this characteristic is especially important in investigations involving polyuse of psychoactive substances, while UNODC

(2023) highlights its wide applicability in both clinical practice and forensic investigation.

When comparing the two methods, Cooper, Kronstrand and Kintz (2019) state that both have high performance when correctly validated. However, LC-MS/MS has been gaining more space in laboratories due to the possibility of multi-residue analyses with less sample preparation and higher productivity, while GC-MS continues to be considered a standard of excellence for several classes of drugs.

To ensure the reliability of the results, SoHT (2023) recommends that all methods be previously validated for selectivity, precision, linearity, limits of detection, and quantification. In addition, UNODC (2023) emphasizes the importance of laboratories' participation in quality control programs and the adoption of international standards, such as ISO/IEC 17025.

Thus, there is a consensus between Levine (2020), Cooper, Kronstrand and Kintz (2019), Musshoff and Madea (2021), Kintz (2023), SoHT (2023) and UNODC (2023) that GC-MS and LC-MS/MS constitute the main technologies of modern forensic toxicology. When associated with standardized laboratory protocols and qualified professionals, these techniques offer high sensitivity, specificity, and reliability, contributing to the production of consistent scientific evidence and the safety of clinical and judicial decisions.

**Limitations of toxicological analysis in hair: external contamination and factors that interfere in the interpretation of the results**

Although toxicological analysis in hair represents an important advance in clinical and forensic toxicology, its use still has limitations that require careful interpretation of the results.

The scientific literature demonstrates that several biological, environmental and methodological factors can influence both the incorporation and the permanence of substances in the hair structure, making any conclusion based exclusively on the qualitative detection of an analyte inadequate. In this context, the interpretation of the exams must consider not only the laboratory aspects, but also the individual characteristics of the examinee, the clinical history, the context of the investigation, and the protocols used during the analysis (Kintz, 2023).

Among the main limitations of hair analysis is the possibility of external contamination. This phenomenon occurs when particles containing drugs or medications adhere to the surface of the hair without the consumption of the substance by the individual. According to Cooper, Kronstrand and Kintz (2019), environments contaminated by illicit drug smoke, industrial dust, aerosols or direct contact with certain substances can favor this type of surface deposition. Thus, the simple identification of a chemical compound in hair does not constitute, in isolation, definitive evidence of ingestion or use of the substance.

The concern with environmental contamination has motivated the elaboration of international protocols aimed at the standardization of toxicological tests. The Society of Hair Testing (SoHT, 2023) recommends that all samples undergo rigorous decontamination procedures prior to instrumental analysis, using standardized washing sequences capable of removing external contaminants without compromising the substances effectively incorporated into the interior of the hair fiber.

However, Kintz (2023) notes that there is still no universal protocol capable of completely eliminating all possibilities of contamination, which is why laboratory results should always be interpreted in an integrated manner.

In addition to external contamination, another widely discussed factor refers to the biological characteristics of the hair. Pragst and Balikova (2006), in a study considered a reference in the area, demonstrated that melanin exerts an important influence on the incorporation of several basic drugs, especially cocaine, amphetamines and opioids. According to the authors, darker hair, because it has a higher concentration of melanin, tends to retain higher amounts of these substances when compared to light hair.

This observation continues to be corroborated by more recent studies. Kintz (2023) highlights that differences related to hair pigmentation can significantly influence the concentrations found, making it necessary to carefully interpret the results obtained.

Another relevant aspect involves the cosmetic treatments performed on the hair. Procedures such as bleaching, coloring, chemical straightening, perm and frequent use of high temperatures can alter the structure of keratin and favor the partial loss of previously incorporated substances. Levine (2020) explains that these structural changes can reduce the concentration of analytes detected during laboratory tests, increasing the possibility of false-negative results or underestimation of the intensity of consumption. Cooper, Kronstrand and Kintz (2019) add that the individual's cosmetic history should be part of the forensic evaluation, as it directly interferes with the reliability of the quantitative analysis.

Hair growth also represents an important variable in the chronological interpretation of the results. Although it is widely accepted that human hair has an average growth of approximately one centimeter per month, Musshoff and Madea (2021) point out that this speed is influenced by genetic factors, age, sex, nutritional status, systemic diseases, and hormonal conditions.

Consequently, the time estimate obtained by the segmental analysis should be considered approximate, and it is not possible to establish exact consumption dates solely based on the position of the substance along the wire.

Another challenge often mentioned in the literature refers to the individual variability of metabolism. According to Levine (2020), differences related to the absorption, distribution, metabolization, and elimination of drugs directly influence the amount of substance available for incorporation into the hair. Thus, individuals subjected to the same consumption pattern may present different concentrations in the laboratory analysis. This observation is reinforced by Kintz (2023), who highlights the impossibility of establishing a linear relationship between the concentration detected in the hair and the amount actually consumed by the individual.

The quantitative interpretation of the exams also deserves special attention. The United Nations Office on Drugs and Crime (UNODC, 2023) emphasizes that high concentrations do not necessarily mean heavier consumption, just as reduced concentrations do not exclude the occurrence of repeated exposure. Factors related to the type of drug, frequency of use, chemical affinity for melanin, hair growth, and laboratory procedures can significantly modify the quantitative results.

Thus, the values obtained must be analyzed together with clinical information, toxicological history and other available expert evidence.

Another aspect discussed refers to the lack of universal standardization of cut-off values. SoHT (2023) establishes recommendations for several substances, but different countries and laboratories still adopt their own interpretation criteria. According to Cooper, Kronstrand and Kintz (2019), this lack of uniformity can generate divergences between reports issued by different institutions, especially in legal proceedings in which small analytical differences can influence relevant decisions.

The chain of custody of the sample is also an essential factor to ensure the reliability of toxicological tests. Levine (2020) highlights that failures in identification, collection, packaging, transportation, or storage can compromise the integrity of the sample and reduce its evidential value. In this sense, UNODC (2023) recommends that all stages of processing be rigorously documented, ensuring complete traceability from collection to the issuance of the expert report.

Therefore, it is observed that the limitations of toxicological analysis in hair do not reduce its importance in clinical and forensic investigation, but highlight the need for multidisciplinary interpretation of the results. The studies by Levine (2020), Cooper, Kronstrand and Kintz (2019), Musshoff and Madea (2021), Kintz (2023), as well as the recommendations of SoHT (2023) and UNODC (2023), converge in stating that hair examination should be understood as a complementary tool within the investigative process.

When associated with validated laboratory protocols, high-precision analytical methods, and careful evaluation of the clinical and forensic context, this test has high potential to contribute to diagnoses, therapeutic monitoring, and the production of reliable scientific evidence in forensic toxicology.

**Challenges in interpreting toxicological results and future prospects**

The interpretation of the results obtained through toxicological analysis in hair is one of the most complex steps of clinical and forensic toxicology. Although technological advances have provided highly sensitive and specific analytical methods, the simple detection of a substance in the capillary matrix does not allow a conclusion on the frequency, intensity or circumstances of consumption in isolation.

Thus, the interpretation of the exams requires a multidisciplinary approach, integrating knowledge of toxicology, pharmacology, forensic medicine, analytical chemistry, and forensic sciences, in addition to a careful analysis of the clinical and investigative context (Levine, 2020).

One of the main challenges refers to the differentiation between passive exposure and effective consumption of the substance. According to Kintz (2023), certain drugs can be deposited on the surface of the hair through environmental smoke, physical contact, or direct manipulation of the substance, without systemic absorption by the individual. This possibility reinforces the need to interpret the laboratory results together with clinical information, the history of the examinee, the circumstances of the collection and other elements of the forensic investigation.

In this sense, Cooper, Kronstrand and Kintz (2019) state that toxicological interpretation should not be limited to the quantitative data provided by the laboratory. The authors point out that similar concentrations can represent completely different situations, depending on the frequency of consumption, the interval between exposure and collection, the physiological characteristics of the individual and the factors related to hair growth. Levine (2020) shares this understanding by highlighting that the laboratory result should be understood as part of a body of evidence and never as an isolated element to support clinical or judicial decisions.

Another aspect widely discussed in the literature concerns the cut-off values used to interpret the tests. These values correspond to the minimum concentrations established for a sample to be considered positive for a given substance. The Society of Hair Testing (SoHT, 2023) recommends specific values for several classes of drugs, seeking to reduce the influence of external contamination and increase the specificity of exams. However, Kintz (2023) notes that there are still differences between protocols adopted by different countries and laboratories, which can result in different interpretations for the same sample.

The United Nations Office on Drugs and Crime (UNODC, 2023) also points out that the absence of complete international standardization represents a challenge for the harmonization of toxicological reports. According to the institution, although several technical recommendations have been published in recent years, there are still differences related to washing methods, extraction, analytical validation, detection limits and interpretative criteria. This variability reinforces the need for continuous development of international standards capable of standardizing laboratory procedures.

Another important challenge refers to the quantitative interpretation of the concentrations found. Musshoff and Madea (2021) state that there is no direct proportional relationship between the amount of drug detected in the hair and the degree of clinical impairment presented by the individual. This is because factors such as metabolism, frequency of use, hair pigmentation, cosmetic treatments, and hair growth speed can significantly modify the concentrations observed. Thus, high levels do not necessarily indicate heavy consumption, just as low concentrations do not exclude repetitive use of the substance.

In addition to biological aspects, technical factors related to laboratory processing can also interfere in the interpretation of the results. Levine (2020) highlights that failures in collection, storage, sample identification, chain of custody, or methodological validation can compromise the reliability of the tests. For this reason, it is recommended that toxicology laboratories operate in accordance with international quality assurance systems, performing internal controls, proficiency tests and periodic validation of the analytical methods employed.

Technological evolution represents, on the other hand, a promising perspective to minimize many of the limitations that currently exist. Kintz (2023) points out that the development of high-resolution mass spectrometry equipment, new chromatographic techniques, and advanced data processing software has significantly increased the ability to simultaneously identify different substances present in the capillary matrix. These innovations make it possible to detect compounds in increasingly smaller concentrations, reducing analytical interference and increasing the robustness of the results.

Cooper, Kronstrand and Kintz (2019) add that advances in metabolomics, proteomics and molecular analysis technologies tend to expand the diagnostic potential of hair toxicology in the coming years. According to the authors, new approaches may make it possible not only to identify the presence of substances, but also to understand metabolic changes related to the pattern of consumption, offering more complete information for clinical and forensic applications.

Another important perspective involves the use of artificial intelligence and computational tools to interpret laboratory results. Recent studies indicate that algorithms capable of integrating clinical, laboratory, and toxicological information can help standardize the interpretation of tests, reducing the currently existing subjectivity and increasing diagnostic accuracy (UNODC, 2023). Although this application is still in the development phase, its potential to strengthen forensic toxicology has been widely recognized by the scientific community.

It is observed, therefore, that toxicological analysis in hair represents a consolidated methodology of high scientific value, but its correct use depends on the integration between technology, technical knowledge and specialized interpretation. The studies by Levine (2020), Cooper, Kronstrand and Kintz (2019), Musshoff and Madea (2021), Kintz (2023), as well as the recommendations of the Society of Hair Testing (2023) and the United Nations Office on Drugs and Crime (2023), demonstrate consensus on the need to interpret the tests in a contextualized way, considering biological, analytical and legal factors.

Thus, the evolution of laboratory techniques, combined with the international standardization of procedures and the continuous improvement of professionals in the area, tends to further strengthen the reliability of toxicological analysis in hair, expanding its contribution to clinical investigation, forensic medicine and the production of scientific evidence in the field of forensic toxicology.

## FINAL CONSIDERATIONS

Hair toxicology analysis has established itself as an important tool in clinical and forensic toxicology due to its ability to identify the prolonged consumption of psychoactive substances, medications and other chemical agents.

Throughout this study, it was found that this biological matrix has significant advantages over conventional matrices, mainly due to the wide detection window and the possibility of retrospective reconstruction of the individual's exposure history. These characteristics expand its applicability in different contexts, including criminal investigations, therapeutic monitoring, abstinence programs, labor forensics, and evaluations related to forensic medicine.

The results of the review showed that hair analysis is a complementary method to tests performed on blood, urine and saliva. While these matrices make it possible to identify recent exposures and assess immediate pharmacological effects, hair makes it possible to document patterns of consumption over weeks or months. In this sense, there was a consensus among the authors analyzed regarding the potential of toxicological analysis in hair to strengthen the production of scientific evidence and support more grounded clinical and judicial decisions.

It was also possible to verify that technological advances, especially with the use of gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), contributed significantly to the increase in sensitivity, specificity and reliability of toxicological tests. These techniques make it possible to identify multiple substances at extremely low concentrations, reducing analytical interference and strengthening the credibility of the reports issued by specialized laboratories.

However, the research also demonstrated that the interpretation of the results remains one of the greatest challenges of contemporary forensic toxicology. Factors such as external contamination, individual biological characteristics, hair pigmentation, cosmetic treatments, hair growth speed and metabolic differences can interfere with the incorporation of substances and modify the concentrations detected. Thus, the mere presence of an analyte in the hair should not be considered isolated evidence of consumption, and the integration between laboratory data, clinical history, investigative context and other expert elements is indispensable.

Another relevant aspect identified refers to the need for greater international standardization of laboratory procedures and the criteria used to interpret the tests. Although entities such as the Society of Hair Testing (SoHT), the United Nations Office on Drugs and Crime (UNODC) and the European Workplace Drug Testing Society (EWDTS) have published important technical recommendations, there are still methodological differences between laboratories and countries, which reinforces the need for constant scientific updating and harmonization of the protocols employed.

In view of this evidence, it is concluded that toxicological analysis in hair represents a tool of high value for the Health Sciences and forensic investigation, as long as it is used judiciously, associated with validated analytical methods and interpreted by qualified professionals. The continuous evolution of analytical technologies, combined with the development of increasingly standardized international protocols, tends to increase the reliability of this methodology and strengthen its application in clinical, forensic and judicial practice. Finally, it is hoped that this study will contribute to expand the knowledge about the potentialities and limitations of toxicological analysis in hair, encouraging new research that promotes the improvement of laboratory techniques and the interpretation of toxicological results.

## REFERENCES

COOPER, G.; KRONSTRAND, R.; KINTZ, P. Society of Hair Testing guidelines for drug testing in hair. Forensic Science International, v. 300, p. 34-43, 2019.

EUROPEAN WORKPLACE DRUG TESTING SOCIETY (EWDTS). European Laboratory Guidelines for Workplace Drug Testing in Hair. London: EWDTS, 2022.

GIL, A. C. Methods and techniques of social research. 7. ed. São Paulo: Atlas, 2019.