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Prospects of Spectroscopy in Forensic Practice: a Review

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ABSTRACT

The article reviews the perspectives of spectroscopy in forensic practice. Spectroscopy is a pivotal analytical tool for the investigation of biological physical evidence. The primary focus of this study is on two major methods: Fourier transform infrared spectroscopy and Raman spectroscopy. Fourier transform infrared spectroscopy is based on the absorption and transmission of infrared radiation by the sample. This approach can be used to determine the molecular composition and chemical bonds in the sample. In contrast, Raman spectroscopy uses laser light scattering to analyze the molecular structure and chemical composition of samples. Both methods are highly precise, fast, and non-destructive, making them vital in forensic medicine. Successful applications of spectroscopy in forensic practice include the identification of various biological fluids such as blood, semen, and saliva. Consequently, Fourier infrared spectroscopy can differentiate between blood types, including peripheral and menstrual, detect specific molecules and determine their concentrations. Meanwhile, Raman spectroscopy has been successfully used to identify the blood of an adult and a newborn. The integration of spectroscopic methods with chemometric approaches and machine learning algorithms is a promising area. This integration facilitates the processing of large amounts of spectra, improves the analytical accuracy, and enables the identification of the test sample. These approaches have been shown to provide more accurate and reliable identification of causes of death and physical evidence.

Consequently, the advanced spectroscopic methods offer fast, accurate and reliable tools for forensic examinations. These methods contribute to the advancement of interdisciplinary teamwork and the introduction of the latest technologies into practice, which leads to the improvement of the quality of forensic examinations and the solution of practical challenges.

Keywords: spectroscopy; forensic medicine; Fourier spectroscopy; Raman spectroscopy; physical evidence; analysis; cause of death; review.

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Перспективы применения спектроскопии в судебно-медицинской практике: научный обзор

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АННОТАЦИЯ

В статье обсуждаются перспективы использования спектроскопии в судебно-медицинской практике. Спектроскопия служит важным аналитическим инструментом для исследования вещественных доказательств биологического происхождения. Основное внимание уделено двум основным методам: инфракрасной Фурье-спектроскопии и рамановской спектроскопии. Инфракрасная Фурье-спектроскопия характеризуется воздействием инфракрасного излучения на образец с последующим анализом спектра поглощения или прохождения света. Этот метод позволяет определять молекулярный состав и химические связи в исследуемом материале. Рамановская спектроскопия, напротив, использует лазерное рассеяние света для анализа молекулярной структуры и химического состава образцов. Оба метода обладают высокой точностью, скоростью и возможностью проведения неразрушающего анализа, что делает их незаменимыми в судебной медицине. Примеры успешного применения спектроскопии в судебной практике включают идентификацию различных биологических жидкостей, таких как кровь, сперма и слюна. Так, инфракрасная Фурье-спектроскопия позволяет различать типы крови, включая периферическую и менструальную, а также определять наличие и концентрацию определённых молекул. В свою очередь, рамановскую спектроскопию успешно применяют для идентификации крови взрослого человека и новорождённого. Важное место занимает интеграция спектроскопических методов с хемометрическими подходами и алгоритмами машинного обучения. Это позволяет обрабатывать большие объёмы спектральных данных, улучшать точность анализа и идентифицировать исследуемые образцы. Такие подходы обеспечивают более точное и надёжное установление причин смерти и идентификацию вещественных доказательств.

Таким образом, современные спектроскопические методы предлагают быстрые, точные и надёжные инструменты для судебно-медицинской экспертизы. Они способствуют развитию междисциплинарного сотрудничества и внедрению новейших технологий в практику, что ведёт к повышению качества судебно-медицинских экспертиз и разрешению сложных практических задач.

Ключевые слова: спектроскопия; судебная медицина; Фурье-спектроскопия; рамановская спектроскопия; вещественные доказательства; анализ; причина смерти; обзор.

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光谱学在法医学实践中的应用前景：科学综述

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简介

文章讨论了光谱学在法医学实践中的应用前景。光谱学是研究生物来源物证的重要分析工具。重点介绍了两种主要方法：傅里叶红外光谱和拉曼光谱。傅里叶红外光谱的特征是将红外辐射照射到样品上，然后分析光的吸收光谱或光透射光谱。这种方法可以确定所研究材料中的分子组成和化学键结。相反，拉曼光谱使用激光散射来分析样品的分子结构和化学成分。这两种方法都具有高精度、快速性和无损分析的能力，从而使它们在法医学中不可或缺。在司法实践中成功应用光谱学的例子很多，包括鉴定各种生物液体，如血液、精液和唾液。因此，傅立叶红外光谱可以帮助区分血型，包括外周血和月经血，以及确定一定分子的存在和浓度。与此同时，拉曼光谱已成功用于识别成人和新生儿的血液。光谱学方法与化学计量学方法和机器学习算法的融合具有重要意义。这有助于处理大量光谱数据、提高分析精度和识别研究样品。这种方法可以更准确和可靠地确定死因和识别物证。

因此，现代光谱技术为法医鉴定提供了快速、准确和可靠的工具。有助于发展综合学科合作，将最新技术引入实践，从而提高法医鉴定的质量并解决复杂的实际问题。

关键词：光谱学；法医学；傅里叶光谱；拉曼光谱；物证；分析；死因；审查。

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INTRODUCTION

Forensic medicine is a multidisciplinary field that combines various concepts and subject areas to reach comprehensive conclusions and support criminal investigations [1]. A careful examination of biological evidence, corpses, images, and other materials may reveal important details, such as the cause of death or the mechanism of injury. This helps ensure fair and accurate judicial decisions.

The development of new technologies has improved our ability to link objects found at a crime scene to specific individuals, which significantly increases the effectiveness of investigations [2–5]. In most cases, biological evidence, such as body fluids or tissues, is found at the scene of the crime [6–8]. These biological materials may be degraded or mixed with other substances, which makes it difficult to detect and identify them [9].

In forensic practice, it is crucial to use quantitative analysis and statistical significance parameters to obtain objective and accurate results. Therefore, special focus should be given to techniques such as Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy. These techniques efficiently detect proteins and nucleic acids, making them effective tools for addressing forensic issues [10, 11].

PRINCIPLE OF FOURIER TRANSFORM INFRARED SPECTROSCOPY

In FTIR spectroscopy, a sample is exposed to infrared radiation, and a resulting absorbance spectrum is processed using Fourier transform-based techniques. This process reliably identifies the chemical bonds and functional groups in the sample. Light beams of different frequencies are directed into an interferometer to collect high-resolution spectral data across a broad frequency range [12]. Data are extracted through the quantitative measurement of light absorbance. As the mirrors move inside the interferometer, light of various wavelengths is periodically blocked or transmitted. The result is an interferogram. The Fourier transform converts the original data obtained for the sample into an absorbance spectrum, which represents light absorbance at different wavelengths [12].

FTIR spectroscopy is based on the interaction between chemical substances and infrared light. The atoms within molecules are in constant motion and vibration. These vibrations are either symmetric or antisymmetric depending on whether the molecules are stretched, deformed, or bent. The vibrations occur at frequencies associated with the chemical bonds and properties of compounds, corresponding to the near-infrared spectrum [13]. Therefore, FTIR spectroscopy can accurately determine the molecular composition of a sample and identify possible abnormal changes, providing valuable diagnostic information [14]. For example, Mader et al. [15] used multidimensional FTIR spectroscopy to evaluate intervertebral disc degeneration.

FTIR spectroscopy is a widely used technique that is often combined with attenuated total reflectance (ATR) to form ATR-FTIR. This combination significantly simplifies the testing procedure and improves the test sensitivity [16]. Therefore, infrared spectroscopy is widely represented in forensic practice. This technique provides a deeper understanding of the composition and properties of test samples, such as skeletal remains, vehicle paintwork, and soil composition [17–19].

PRINCIPLE OF RAMAN SPECTROSCOPY

Raman spectroscopy is an analytical technique that uses the interaction of light with a sample and the changes in the charge distribution of the sample's molecules under laser radiation [20]. This interaction results in an exchange of energy and momentum, which is expressed as Rayleigh and Raman scattering. In most cases, light interacts with molecules in a way that only changes its direction without altering its energy. This is called elastic or Rayleigh scattering. However, when a molecule absorbs energy from incident light, it starts to vibrate, resulting in a difference in energy between the scattered and incident light. This process is called inelastic or Raman scattering [21].

Changes in laser energy are associated with collisions with different molecules in the sample, leading to transfer of varying amounts of energy. As a result, each molecule demonstrates unique characteristics that appear as peaks in the Raman spectrum. Raman spectroscopy collects inelastic scattered tissue-specific light, creating a molecular fingerprint of that tissue [22]. This technique is used for the qualitative and quantitative analysis of samples. By evaluating a sample's structure, composition, and chemical bonds, it accurately characterizes the vibrations of specific chemical molecules [23].

In practice, Raman spectroscopy can be combined with other techniques to provide the following:

- Surface-enhanced Raman spectroscopy
- Stepwise Raman spectroscopy
- Micro-Raman spectroscopy

Fiber Raman spectroscopy is shown to rapidly and accurately diagnose muscle diseases in humans [22, 24, 25]. In addition, the numerous advantages of Raman spectroscopy have led to its widespread use in forensic medicine, providing valuable data for analytical tests and investigations [26, 27].

FTIR and Raman spectroscopy are considered the primary techniques used to evaluate molecular vibration modes. FTIR spectroscopy evaluates changes in molecular dipole moments, whereas Raman spectroscopy evaluates changes in polarizability [28]. Both techniques are significant tools for non-destructive measurements, offering a wide spectral range, ease of operation, and minimal sample preparation requirements [29–32].

Biological material is carefully selected and fixed to prevent destruction and contamination. Handheld devices, such as handheld FTIR and micro-Raman spectrometers, allow analysis to be performed directly at the scene. Stationary devices, including laboratory FTIR and surface-enhanced Raman spectrometers, provide more detailed analysis in a laboratory setting [22, 24, 25].

A non-destructive analytical technique is based on heating without combustion. The heating temperature is typically approximately 50–60°C, which is sufficient to activate the processes without causing denaturation of most proteins [29–32]. There is a risk of denaturation, but it is minimal as long as the required temperature conditions are met.

Therefore, the proper technical equipment should be used and strict protocols for preparing and evaluating biological materials should be followed to minimize the risk of damaging samples and losing valuable data that could be used in future investigations.

CHEMOMETRICS

Chemometrics is a powerful experimental data-based analytical tool used in chemistry to extract valuable information from measurement results by leveraging mathematical and statistical methods. Chemometrics involves processing of data in matrix form to identify potential relationships between variables, thereby simplifying the analysis of the multivariate characteristics in forensic investigations [33]. The widely used MATLAB, as well as Python and R programming languages, provide efficient and accurate chemometric analysis.

Preprocessing is usually required to prepare spectral measurement data for classification or calibration. This process can significantly improve and systematize the obtained results. In addition, preprocessing can effectively eliminate or minimize noise and interference in the spectrum, improving the predictive performance of the model [34, 35]. Common spectral preprocessing techniques commonly include smoothing, baseline correction, and scatter correction [36]. These techniques produce more accurate and reliable analytical results, which are crucial to effectively use chemometrics in forensic medicine.

Chemometric models can be supervised and unsupervised. Unsupervised models are built without predefined labels and are intended for research purposes. They identify data trends, group the similar data points together, and separate different data sets. Clustering is a key method for identifying hidden structures in a dataset. The most widely used clustering techniques include hierarchical clustering, K-means clustering, and principal component analysis (PCA). Supervised models are trained using human-labeled input data and grouped data. They are used to categorize new samples based on known patterns. Common supervised learning techniques include partial least squares discriminant analysis (PLS-DA), linear discriminant analysis, and support vector machines. These techniques extract valuable features and achieve high classification accuracy [37].

Stoichiometry establishes a statistical basis for spectral analysis, allowing for the comparison of a sample's spectrum to a standard chemometric model [21]. Many studies highlight that the synergetic use of Raman and FTIR spectroscopy in combination with chemometric techniques can significantly improve the accuracy and reliability of forensic conclusions [38–41].

Biological Evidence

Locard's exchange principle states that when a person comes into contact with an object or another person, a cross-transfer of physical evidence occurs [42]. This statement emphasizes the importance of identifying biological evidence at the crime scene to draw initial conclusions about the suspect's phenotype. The effective identification and recovery of relevant physical evidence is crucial for generating information that will help reduce the pool of potential suspects. These techniques facilitate investigative activities. Forensic investigations often rely on biological fluids as a primary source of evidence because these substances are commonly found at crime scenes. Their analysis is key to determining various features and significantly contributes to reconstructing events [43]. However, many analytical techniques are destructive, and available samples are limited. Therefore, non-destructive sampling is necessary for accurate and efficient identification during investigations. Spectroscopy-based identification techniques are considered the optimal solution for evaluating biological evidence. Raman and FTIR spectroscopy are becoming more common in this approach because of their high sensitivity. These techniques identify and preserve samples for future testing.

Blood Stains

Blood is the most common biological fluid found at violent crime scenes, so many studies aim to find the optimal method for detecting it. For example, Sharma et al. [44] conducted a comprehensive analysis using ATR-FTIR spectroscopy. Their study included 50 samples of venous blood from healthy individuals, 30 samples of menstrual blood, 30 samples of semen and vaginal fluid, and various non-biological substances that can mimic blood and produce false-positive results. A blood stain was applied to the surface of a crystal used for ATR spectroscopy. The combined use of chemometric techniques, such as PCA and linear discriminant analysis, classified blood and other body fluids, as well as blood-like non-biological substances with the 100% accuracy. Moreover, ATR-FTIR was shown to reliably determine the age of bloodstains on days 1, 7, and 15 on a glass slide.

Fujihara et al. [45] conducted a similar study that demonstrated the ability to distinguish between infant and adult blood stains using micro-Raman spectroscopy. Forensic practice requires the accurate identification not only of bloodstains but also of their species. For example, in the case of a traffic accident, the ability to distinguish between human and animal blood can play a crucial role in the investigation and determination of the incident circumstances. Many researchers are exploring the combined use of vibrational spectroscopy and chemometrics to identify blood species. Takamura et al. [46] presented an innovative trapezoidal Raman spectrometer with surface-enhanced Raman spectroscopy capabilities. They developed a comprehensive spectral database that covered 26 species, including humans. The researchers achieved an identification accuracy rate of over 94% using convolutional neural network algorithms, which proved effective and reliable for identifying blood species [47].

Many other studies also evaluate spectroscopic techniques. For example, Kumar et al. [48] used ATR-FTIR spectroscopy to analyze infrared spectra of bloodstains and developed a model using partial least squares regression to measure their formation time. This made it possible to reliably determine the age of a bloodstain within a range of 1 to 175 days. Gautam et al. [49] demonstrated the reliability and effectiveness of Raman spectroscopy using the least absolute compression and feature selection to determine the age of bloodstains on different surfaces, such as floor tiles and linoleum, ranging from 1 to 336 hours. The input power (2 MW) and exposure time (15 seconds) were optimized to prevent unwanted heating of or damage to the blood sample.

Therefore, current spectroscopic techniques such as ATR-FTIR and Raman spectroscopy demonstrate high potential for solving many forensic issues, including blood identification, estimation of bloodstain formation, and determination of blood species. The combination of spectroscopic and chemometric techniques is essential for reaching more accurate and reliable conclusions; therefore, they are considered indispensable tools in the arsenal of modern forensic experts.

Semen Stains

Semen is often found at crime scenes, especially in sexual assault cases. Zha et al. [50] placed sperm samples on three different substrates and analyzed them using ATR-FTIR spectroscopy in combination with chemometric techniques, such as PCA and partial least squares. This approach reliably estimated the time of sperm stain formation *in vitro*, ranging from 0.5 to 6 days. Investigators can use this data to verify alibis, determine the time and date of a crime, and estimate the time of death.

Semen species should also be determined. Wei et al. [51] successfully applied classification models obtained using PLS-DA and FTIR spectroscopy to identify humans and species, such as rabbits, dogs, pigs, cows, and sheep, achieving 100% predictive accuracy. This highlights the practical value of vibrational spectroscopy as a tool for identifying semen species in real-world forensic investigations. Notably, this approach to species identification does not require whole sperm cells, which makes it flexible and suitable for analyzing small quantities or partially destroyed material.

Saliva

Saliva is a promising biological material for analysis because its composition closely resembles that of blood. Al-Hetlani et al. [52] analyzed 32 saliva samples using Raman spectroscopy. The authors developed an artificial neural network model that could classify smokers and nonsmokers with 100% accuracy during external validation. Buchan et al. [53] systematically evaluated the molecular spectral fingerprint of saliva using a combination of Raman spectroscopy and a hybrid artificial neural network algorithm. The researchers used the spectral fingerprints of saliva to stratify participants into the following age groups:

- Young adults (20–30 years)
- Middle-aged adults (31–55 years)
- Older adults (>56 years)

In addition, they stratified the participants by sex in order to identify unique molecular features in women and men. Saliva is a promising material for forensic purposes because its composition did not change significantly during a week.

CAUSE OF DEATH

A forensic autopsy relies heavily on identifying macroscopic and microscopic presentations, and this limits the ability to diagnose functional injuries, such as sudden cardiac death, hypothermia, and poisoning [54]. Spectroscopy is a non-destructive analytical technology that can be used on biological samples without prior labeling. This opens up new opportunities for forensic pathologists.

Pulmonary Edema

Lin et al. [55] demonstrated the need for isolating lung interstitial fluid in cases of pulmonary edema to establish the cause of death. The researchers used FTIR spectroscopy to measure the infrared spectrum of interstitial fluid and then integrated this data with a deep learning system based on evolutionary neural networks to create a classification model. Sensitivity and specificity of the model ranged from 0.9661 to 0.9856 and from 0.8774 to 0.9167, respectively. These findings suggest that the combined use of FTIR spectroscopy with deep learning algorithms could create an effective new diagnostic tool for common causes of death, such as cardiomyopathy, carbon monoxide poisoning, and brain hemorrhage.

In forensic practice, it can be challenging to clearly identify fatal anaphylactic shock as the cause of death because it requires an accurate differential diagnosis. Forensic pathologists often need to evaluate subtle morphological and physiological differences, so the lack of relevant information can prevent them from reaching a clear conclusion [56]. Forensic pathologists have reported an increased volume of interstitial fluid in cases of fatal anaphylactic shock [57]. Some authors have also noted differences in lung fluid composition in different causes of edema [58, 59]. Lin et al. [60] evaluated the potential use of lung interstitial fluid to establish cause of death.

They used a combination of FTIR spectroscopy and PCA to identify characteristic differences in the composition of proteins in the interstitial fluid that forms in the lungs during anaphylactic shock. The researchers discovered differences in the biochemical composition of interstitial lung fluid between cases of fatal anaphylaxis and cases of death due to other causes, including mechanical asphyxia, traumatic brain injury, and acute heart failure. Infrared spectroscopy and pattern recognition techniques revealed higher levels of proteins, such as albumin and globulin, in interstitial lung fluid associated with anaphylactic pulmonary edema. Analysis of the secondary structure of the proteins revealed a higher percentage of α-helices and turns, as well as a lower percentage of tyrosine-rich proteins compared with the control group. These findings were supported by a PLS-DA model, which precisely classified cases of anaphylactic shock. Therefore, PLS-DA could be used to diagnose fatal anaphylaxis postmortem.

Sudden Cardiac Death

In forensic medicine, differential diagnosis is most often required in cases of sudden death, which may be caused by cardiovascular disease, including sudden cardiac death [54, 61]. This condition is common in patients who appear to be healthy. It is characterized by sudden onset and rapid progression, leading to death. In some cases, a standard autopsy is not enough to determine the cause of death, and additional tests are required for accurate identification. Traditional autopsy and microscopic techniques often fail to distinguish between asphyxia and sudden cardiac death due to the absence of the specific morphological signs. Zhang et al. [62] conducted experiments using FTIR spectroscopy combined with a support vector machine to evaluate biochemical differences in lung tissue obtained from rats and humans who died from asphyxia or sudden cardiac death. The researchers found that rats that died from asphyxia had higher levels of lipids and proteins in their lung tissue than rats that died from sudden cardiac death. These differences were confirmed by data obtained 24 hours after the animals died, supporting that this technique can determine the cause of death even after tissue decomposition begins. In addition, seven of the nine identified differential spectral features were found to be significant in human lung tissue samples, meaning that this approach could be used in forensic practice to diagnose causes of death [62].

Empirical data show that myocardial fibrosis is a common manifestation of sudden cardiac death. Therefore, identifying myocardial fibrosis is a promising new method for diagnosing sudden cardiac death. This conclusion was confirmed by Yang et al. [63]. The researchers obtained the infrared spectrum of heart tissue using ATR-FTIR spectroscopy and then evaluated it using PLS-DA. A total of 129 tissue blocks taken from human hearts were examined using ATR-FTIR spectroscopy and hematoxylin and eosin staining. The samples were divided into the experimental group (with myocardial fibrosis) and the control group (without myocardial fibrosis). The chemometrics classification results showed that the sensitivity and specificity of the training dataset were 0.91 and 1.0, respectively, and the sensitivity and specificity of the predictive dataset were 0.862 and 0.900. This study demonstrated that ATR-FTIR spectroscopy combined with chemometrics is an effective method for identifying myocardial fibrosis.

Sudden cardiac death is often associated with myocardial infarction. Spectral analysis revealed distinctive features for each stage, and this finding was confirmed by the PCA. An automated classifier based on artificial neural networks successfully recognized these stages, and visualization using pseudocolor images showed high agreement with histological data. This approach has been shown to effectively and objectively assess the histological stage of myocardial infarction in forensic practice [64]. FTIR spectroscopy has also been shown to effectively detect tissue indicative of early myocardial ischemia, even when no morphological changes are observed [65].

Drowning

Drowning is a type of mechanical asphyxia caused by obstruction of the airways and alveoli, which leads to impaired gas exchange, insufficient oxygen supply, and carbon dioxide buildup in the body. In forensic practice, drowning is usually confirmed by the detection of planktonic diatoms [62, 66, 67]. However, it is often absent in people who died from drowning and may be found in high concentrations in people who died from other causes after spending a long time in a diatom-rich environment. This complicates the diagnostic process because of the need to accurately distinguish between drowning and postmortem submergence. Different postmortem submergence intervals may result in different concentrations of planktonic diatoms, requiring precise identification of these factors [68]. Xiong et al. [70] evaluated the infrared spectrum of lung tissue samples using FTIR spectroscopy combined with PLS-DA. The researchers found that significant differences in amide I and amide II levels could effectively distinguish cases of drowning from postmortem submergence. In addition, PCA revealed differences between samples exposed to freshwater and saltwater, which were associated with varying concentrations of the drowning medium. Notably, the degree of lung tissue degradation did not significantly impact the conclusions. PLS-DA-based models have been developed for identifying drowning and postmortem submergence in both fresh and saltwater environments. Both models demonstrated high classification accuracy, achieving 94.4% and 100%, respectively. Averaged second-derivative spectra and chemometric techniques revealed that differences in protein structure and content are the primary factors that distinguish drowning from postmortem submergence cases. This pilot study demonstrated the effectiveness of using ATR-FTIR spectroscopy combined with chemometrics for forensic drowning diagnosis.

EXPOSURE TO EXTREME TEMPERATURES

In practice, it is often necessary to differentiate between sudden death and hypothermia. Ischemic heart disease, brain hemorrhage, and other conditions often develop at low temperatures, making it difficult to differentiate them from deaths caused directly by hypothermia. Although abnormal changes in the cardiovascular system during hypothermia have been well studied, the exact role of these changes in diagnosing thanatogenic processes is not fully understood [70].

Lin et al. [71] demonstrated the effectiveness of using FTIR spectroscopy combined with chemometrics to detect pulmonary edema as a marker specific to fatal hypothermia. A comparative analysis of spectral profiles revealed that patients with pulmonary edema who died from hypothermia had lung fluid with more β-sheet protein structures than patients who died from other causes. A PLS-DA-based postmortem diagnosis model for fatal hypothermia accurately identified the causes of death in eight new cases. These findings suggest the potential for using FTIR spectroscopy in combination with chemometrics to diagnose fatal hypothermia postmortem.

The hypothalamus plays a key role in regulating body temperature, and its metabolism and functional activity also change in response to internal temperature fluctuations. Lin et al. [72] used FTIR spectroscopy and the random forest method to evaluate the infrared spectrum of the hypothalamus in order to determine the effects of fatal hypothermia and hyperthermia. The study showed that fatal hyperthermia was associated with increased total lipid content, decreased levels of unsaturated fatty acids, and impaired cell membrane mobility. However, significant increases in protein aggregation abnormalities and nucleic acid levels were observed in fatal hypothermia. These results suggest that FTIR spectroscopy is an effective method for evaluating the biochemical properties of hypothalamus under extreme temperatures. A similar experiment evaluated lethality models under hyperthermia and hypothermia by analyzing plasma using ATR-FTIR spectroscopy combined with PLS-DA. The study revealed lower levels of total lipids and long-chain fatty acids in cases of fatal hyperthermia, as well as higher levels in cases of fatal hypothermia, compared with the control group. In addition, fatal hyperthermia was associated with the highest levels of unsaturated lipids, whereas fatal hypothermia was associated with the highest levels of carbonyl ester [73]. Previous studies have demonstrated the potential of using metabolomics to diagnose fatal hypothermia by analyzing the composition of the vitreous body. Spectroscopy, which yields results comparable to metabolomics data, determines the composition of test samples and is suitable for analyzing liquid materials. Therefore, further research can evaluate the potential use of spectroscopy to identify vitreous components [74]. The combined use of metabolomics and spectroscopy techniques can significantly improve the accuracy and reliability of diagnosing fatal outcomes due to exposure to extreme temperatures, which is crucial for forensic practice. Such research could lead to new techniques for identifying cases of hypothermia- and hyperthermia-related death more effectively based on chemical and biochemical analysis. This could help address practical challenges.

Diabetes Mellitus and Its Complications

Diabetic ketoacidosis can be fatal and complicate the determination of cause of death during a routine autopsy due to the absence of characteristic morphological changes [75]. Wu et al. [76] used FTIR spectroscopy to evaluate interstitial lung fluid obtained from corpses. The study used PLS-DA to create a classification model. The results revealed significant changes in the proteins found in the interstitial fluid of patients with diabetic ketoacidosis, supporting the potential use of FTIR for diagnosing and detecting this condition.

In addition, a clear bidirectional correlation has been established between diabetes mellitus and heart failure, with diabetic cardiomyopathy being the primary cardiac manifestation of diabetes mellitus [77]. Most diagnostic techniques aim to identify late stages, whereas studies of the early stages, when there are no obvious morphological or functional changes to the myocardium, are limited. However, FTIR technology has shown potential for diagnosing diabetic cardiomyopathy in forensic practice. Carbonyl esters, alkyne groups, and CH and CH2 lipids were identified in the myocardium of diabetic mice. Significant changes in the conformational transformation of the α-helixes and β-sheets of total lipids, sugars, and proteins were reported compared with healthy mice. These data suggest the potential use of FTIR spectroscopy to confirm or exclude death due to diabetic cardiomyopathy during an autopsy [78]. A similar study used ATR-FTIR spectroscopy to evaluate various body fluids in a mouse model of diabetic cardiomyopathy. The results revealed a linear correlation between disease severity and biomarker levels in plasma, saliva, urine, and a mixture of plasma and saliva. These data highlight the potential of ATR-FTIR spectroscopy for the rapid diagnosis of diabetic cardiomyopathy [79].

Spectroscopy is a unique chemical analysis technique used to determine the composition and molecular structure of human tissue and other substances. This approach provides the comprehensive and valuable information required to determine the cause of death in a forensic investigation. Unlike traditional analytical techniques, which require complex sample preparation and separation, spectroscopy can evaluate untreated samples directly, thereby optimizing the process.

Spectroscopic techniques are faster and more accurate and reliable tools for forensic investigations involving corpses and biological evidence. The integrated use of machine learning algorithms with various analytical technologies is expected to improve data analysis accuracy and test sample identification, advance current forensic techniques, and promote interdisciplinary collaboration.

DIRECTIONS FOR FUTURE RESEARCH

Raman and FTIR spectroscopy are two major analytical techniques with significant practical potential and many avenues for further development in forensic medicine. In FTIR spectroscopy, a sample is exposed to infrared radiation, the amount of light absorbed or transmitted is measured and converted into a spectrum that reveals the molecular composition of the sample. This technology is invaluable to forensic medicine because it can detect evidence too small to be seen by the naked eye. It is highly effective at distinguishing between different types of bloodstains, such as those from peripheral and menstrual blood, as well as at detecting the presence and concentration of specific molecules. FTIR spectroscopy is notable for its ability to provide biochemical profiling of the hypothalamus during fatal hyperthermia or fatal hypothermia.

Raman spectroscopy, in turn, measures scattered light rather than absorbed or transmitted radiation. This approach uses inelastic photon scattering to determine the molecular composition of a sample and identify its chemical bonds. Raman spectroscopy, for example, can accurately distinguish between adult and infant bloodstains.

Both Raman and FTIR spectroscopy are invaluable, non-destructive tools in forensic medicine that maintain the integrity of samples for further investigation, if necessary. Both techniques are valuable and important tools that are rapidly evolving in terms of technology and practical application. Technological advances in Raman and FTIR spectroscopy can increase sensitivity, reduce detection time, and improve overall efficiency.

In addition, new software and algorithms are being developed to synergistically integrate these technologies with vibrational spectroscopy, thereby improving the accuracy and reliability of the obtained data. Further miniaturization and automation of Raman and FTIR spectroscopy are expected to make these technologies more practical and convenient. These techniques will continue to be applied more widely in forensic medicine, providing new opportunities to improve the quality of investigations.

CONCLUSION

Raman and FTIR spectroscopy are highly effective analytical techniques that are widely used in forensic medicine to solve many diagnostic challenges, such as the identification of biological evidence and the determination of causes of death. Both techniques are characterized by their high level of accuracy, short turnaround time, and potential for non-destructive analysis, making them promising tools for use in forensic medicine.

However, the implementation of Raman and FTIR spectroscopy is limited by their high cost, despite their effectiveness. Moreover, forensic and anatomic pathology involves comprehensive spectral data analysis, which often requires expertise in machine learning and artificial intelligence. The lack of this knowledge presents an additional challenge.

As our society and artificial intelligence technologies continue to evolve, the barriers to implementing Raman and FTIR spectroscopy are expected to decrease, leading to the wider use of these techniques in forensic practices.

ADDITIONAL INFORMATION

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