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Review

Vibrational Spectroscopy in Forensic Science: A New Frontier for Biopharmaceutical Drug Authentication

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ABSTRACT: The global proliferation of counterfeit biologic medicines poses a growing threat to public health and pharmaceutical integrity. Traditional laboratory-based methods for verifying drug authenticity are often time-consuming, costly, and impractical for real-time or field-based applications. This paper explores the emerging potential of infrared (IR) and Raman spectroscopy for forensic detection and authentication of biologics. While these technologies are currently underutilised in forensic science, advancements in instrumentation and data analysis are rapidly enhancing their sensitivity, portability, and usability. Focusing on protein- and peptide-based therapeutics, the paper reviews the principles and applications of IR and Raman spectroscopy, highlighting their ability to detect structural and compositional differences between authentic and counterfeit biologic drugs. The discussion emphasises the importance of interdisciplinary collaboration between forensic and biopharmaceutical sciences. As counterfeiters become more sophisticated, the integration of non-destructive spectroscopic tools into forensic workflows offers a promising path toward the rapid and reliable screening of biologic drugs in both field and laboratory settings.

Keywords: Counterfeit; Biopharmaceutical; Infrared; Raman

1. Introduction

According to the World Health Organisation (WHO), countries spend more than 30 billion U.S. dollars annually on substandard and falsified medical products, with low- and middle-income countries being particularly affected, as an estimated 10% of medicines in these regions are substandard or falsified [1]. A 2017 WHO report outlined the significant health, economic, and societal consequences of these products, including increased disease prevalence, mortality, antimicrobial resistance, diminished public trust in healthcare systems, and substantial financial burdens on individuals and health systems [2]. A report published in 2020 by OECD (Organisation for Economic Co-operation and Development) examined the global traits in counterfeit pharmaceuticals and highlighted the associated economic, health, and societal impacts. Among the various interesting findings of the OECD study was that India and China are the largest producers of counterfeit medicines, while Singapore and Hong Kong (China) serve as major transit points. The medicines are then primarily shipped to Africa, Europe, and the United States via express courier and small postal parcels [3].

Given the transnational nature of counterfeit operations, international cooperation is essential. Falsified medicines and medical devices often originate from multiple countries, making it difficult for any single nation to address the issue in isolation. This need for global coordination is exemplified by "Operation Pangea", an annual initiative led by INTERPOL since 2008, which targets the online distribution of counterfeit medicines [4]. Increasing public awareness is equally important, as educating the public about the risks associated with purchasing medicines and medical devices from unverified sources, empowers individuals to make informed decisions. In the UK, the FakeMeds campaign, launched in 2016 by the Medicines and Healthcare products Regulatory Agency (MHRA), aims to raise awareness about the dangers of buying counterfeit medicines and medical devices online, helping people to identify legitimate sources and protect their health. The campaign provides information on how to identify a registered website or pharmacy in the UK and provides guidelines on how to spot a suspicious website as well as a fake medicine or medical device [5].

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Similar initiatives also take place in other countries such as the "BeSafeRx: Know Your Online Pharmacy" by the Food and Drug Administration (FDA) in the U.S. [6], or the EUvsFakeMedicines campaign, a joint campaign between the European Union Intellectual Property Office (EUIPO), Europol and European Medicines Agency (EMA) [7].

Although the term "falsified" is typically associated with public health concerns and "counterfeit" with intellectual property violations [8], various terms have been used interchangeably, and often incorrectly, over the years to describe medicines that have been fraudulently produced and sold. The World Health Organisation has revised these definitions multiple times to improve clarity [2]. For the purposes of this paper, the term "counterfeit" will be used to refer collectively to substandard, falsified, fake, spurious, unregistered, and unlicensed medicines. Such counterfeits may contain the wrong active ingredient, no active ingredient, incorrect dosages, or harmful contaminants such as chemicals, toxins, or microbes [9].

The majority of counterfeit drugs circulating globally are small-molecule therapeutics, often falling into specific high-demand categories. These include lifestyle drugs such as erectile dysfunction and pain medications, benzodiazepines, and synthetic cannabinoids [10]. While small-molecule drugs continue to dominate the landscape of pharmaceutical counterfeiting, there is growing concern about the falsification of biotherapeutics. These biologics, which include monoclonal antibodies, hormone analogues, and recombinant proteins, are often more complex, expensive, and sensitive to manufacturing conditions than traditional small molecules. Their increasing clinical relevance and commercial value have made them attractive targets for counterfeiters, as evidenced by recent alerts and case reports involving falsified biologic products across multiple regions. However, while the issue is occasionally mentioned in broader discussions of counterfeit medicines, dedicated academic studies on counterfeit biologics remain scarce. A search in Google Scholar, Scopus, and Web of Science using keywords such as "counterfeit biologics", "falsified biologics", "substandard biopharmaceuticals", and related terms, yielded fewer than 20 publications over the past five years (2020–2025). This gap underscores the need for further research to address the growing public health concern posed by counterfeit biologics and to develop robust analytical strategies for their detection.

The following examples illustrate forensic alerts related to counterfeit protein- and peptide-based therapeutics. These cases are not intended to be exhaustive but serve to highlight the diversity of biologic modalities affected, including GLP-1 receptor agonists, botulinum toxins, hormones, and monoclonal antibodies. Other biotherapeutic products, such as vaccines, growth factors, and gene therapies, have also been subject to counterfeiting in recent years; however, these modalities fall outside the scope of this paper.

2. Illustrative Cases of Protein- and Peptide-Based Biological Drug Counterfeiting

2.1. GLP-1 Receptor Agonists

Semaglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist used to treat Type II diabetes but has increased in popularity due to its effectiveness in promoting weight loss and treating eating disorders [11]. Due to its increasing popularity and high demand, the drug (which is being sold under commercial names such as Ozempic, Wegovy, Rybelsus) has resulted in counterfeit versions appearing worldwide in recent years. Similarly, liraglutide, another GLP-1 agonist approved for chronic weight management and sold under the brand name Saxenda, is increasingly counterfeited. Recent cases of counterfeit Ozempic were found in the U.S. drug supply chain, which also included counterfeit needles, raising concerns about sterility and additional safety risks [12]. Counterfeit semaglutide products have also been detected in Brazil and Northern Ireland [13]. In 2023, in the UK, MHRA reported that pre-filled pens falsely labelled as Ozempic were found at two UK wholesalers who had obtained the products from legitimate suppliers in Austria and Germany [14]. In addition, counterfeit pens falsely labeled as Saxenda were also detected in the same year. The agency has alerted the public to the potential serious implications of using these products, as some of them may contain insulin [15].

2.2. Botulinum Toxins

Botulinum Toxin Type A (BoNTA), a highly potent neurotoxin derived from the anaerobic bacterium Clostridium botulinum, is known for its ability to induce muscle paralysis. It is considered one of the most lethal biological substances known to science and has been classified as a potential bioweapon. However, when administered in controlled, therapeutic doses, it has demonstrated significant efficacy in treating a range of neuromuscular disorders and has gained widespread use in aesthetic medicine [16]. Several counterfeit Toxin A products have been obtained and tested in the past [17], revealing that many do not match the potency levels claimed on their labels. Some samples showed almost no biological activity, while others, suspected to be of Chinese origin, contained excipients such as

gelatin and sucrose, consistent with older Chinese biologics standards. A particularly concerning finding was that some of these look-alike products exhibited higher-than-labeled potency, which poses serious safety risks. The cases of iatrogenic botulism are on the rise, with various cases being reported in recent years related to adulterated toxin injections [18–21]. Early detection of botulism and administration of the antitoxin are crucial as botulism progresses rapidly and can lead to respiratory failure, muscle paralysis, and death [22]. These recent incidents highlight the growing public health threat posed by BoNTA counterfeit cosmetic products and underscore the urgent need for their detection before they reach patients.

2.3. Hormones

Erythropoietin (EPO) is a glycoprotein hormone that regulates red blood cell production, with recent evidence indicating it acts far beyond erythropoiesis [23]. Its recombinant form (rhEPO) is widely used to treat various forms of anemia, and is also misused as a performance-enhancing drug in sports [24]. EPO has been targeted by counterfeiters, resulting in serious health implications for patients. In the early 2000s, in Florida US, counterfeiters relabeled 2000-unit vials of rhEPO as 40,000 units by affixing counterfeit labels and distributed them through gray market channels, resulting in widespread patient exposure. Investigations by local authorities resulted in the conviction of 17 individuals for their roles in trafficking counterfeit drugs. Despite the scale of the operation, the FDA received only a dozen reports of patients exposed to the counterfeit medication, with detailed clinical information available for just two cases where patients experienced severe side effects following administration of the counterfeit drug [25]. A more recent case of EPO counterfeiting involved an athlete claiming sabotage after testing positive for rhEPO, raising concerns about disguised EPO syringes circulating on the black market [26].

Somatropin, the recombinant form of human growth hormone (hGH), is another biologic frequently counterfeited. Approved by the FDA for conditions such as pediatric and adult GH deficiency, Turner syndrome, and chronic renal insufficiency, somatropin offers clinical benefits, including improved linear growth in children and enhanced body composition in adults [27]. However, its off-label use in sports for muscle growth, fat metabolism, and recovery [28] has made it a target for illegal distribution and counterfeiting. A 2017 study investigated the online availability and pharmaceutical quality of somatropin sold without prescription through illegitimate Internet pharmacies. Researchers identified 17 vendor websites and purchased three samples of somatropin for analysis. Analytical testing using capillary electrophoresis and mass spectrometry revealed that all samples contained significantly lower concentrations of somatropin than labeled, and some included unknown protein components, suggesting degradation or contamination [29]. In March 2025, U.S. Customs and Border Protection (CBP) intercepted 71 illicit shipments during a multi-port enforcement operation, including 67 shipments of counterfeit human growth hormones and steroids. The operation targeted high-risk shipments across mail, express consignment, and air cargo environments at five major ports and two international airports. Most of the seized shipments originated from Hong Kong [30].

2.4. mAbs

Bevacizumab (sold under the commercial name Avastin, Genentech, South San Francisco, CA, USA) is a humanised monoclonal antibody (mAb) that acts as an angiogenesis inhibitor targeting vascular endothelial growth factor (VEGF), and is used to treat various types of cancer, including colorectal cancer [31]. Due to its high cost and widespread clinical use, Avastin has historically been a target for counterfeiters. One of the most well-documented cases of biologic drug falsification involved counterfeit Avastin entering the U.S. drug supply chain in 2012. These falsified products contained no active pharmaceutical ingredient (API) and instead included substances such as cornstarch, salt, and acetone. The incident revealed significant vulnerabilities in the global pharmaceutical supply chain and led to nearly 1000 FDA safety notifications that were sent to physicians across 48 states [32]. While the 2012 incident remains a seminal case in counterfeit biologics, recent events suggest that the threat persists. In 2024, the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC) issued a public alert confirming two counterfeit batches of Avastin [33]. Other recent cases of mAb counterfeiting involved the drug durvalumab, which is used for Non-Small Cell Lung Cancer (NSCLC) in adults. WHO issued alerts in both 2024 and 2025 for the counterfeits that contained no active pharmaceutical ingredient, which were detected in Armenia, Lebanon, Türkiye and the Islamic Republic of Iran [34,35]. These cases highlight the ongoing global circulation of counterfeit mAb therapeutics, demonstrating that the threat is not confined to any particular region or economic tier.

3. Analytical Techniques Commonly Used for the Identification of Biopharmaceutical Counterfeits

Unlike small molecules, biologics present unique challenges for detection due to their structural complexity and formulation variability, necessitating more sophisticated analytical approaches. Most current techniques used to verify the identity and potency of these drugs are lab-based, involving time-consuming processes for sample preparation and data analysis, and equipment that requires highly skilled, specialised personnel. Additionally, transporting samples to certified laboratories increases both the time and cost involved, and by the time authenticity is confirmed, the product has already been distributed to consumers. Basic protein mass spectrometry analysis can provide information on primary sequence and post-translational modifications [36]. More advanced structural MS techniques can provide information on the higher order structure of proteins such hydrogen deuterium exchange MS, ion mobility, or native MS [37–39]. A comprehensive review of case reports from 2010 to 2016 on the analysis of falsified biotherapeutics [40] reveals that liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was the most frequently employed technique in forensic laboratories, followed by SDS-PAGE. There is limited use of techniques such as capillary electrophoresis and ELISA, and vibrational spectroscopy methods, including infrared (IR) and Raman spectroscopy, remain underrepresented, despite their potential for rapid, non-destructive screening of biopharmaceuticals. While it has been previously suggested that screening of primary and secondary packaging, as well as vials, offers a relatively straightforward approach to identifying counterfeit protein-based medicines [41], it is equally important to employ rapid analytical techniques to assess the authenticity and quality of the active pharmaceutical ingredient. This enhances the reliability of detection by not only verifying external packaging integrity but also confirming the biochemical composition of the protein-based therapeutic itself.

Given the limitations of conventional laboratory-based techniques, particularly their lack of portability and real-time applicability, there is a growing need for analytical tools that can support rapid, non-destructive screening of biologic drugs. Vibrational spectroscopy methods, including infrared (IR) and Raman spectroscopy, offer promising solutions in this regard. These techniques are already widely used in biopharmaceutical development, yet remain underutilised in forensic science. The following sections explore the principles, applications, and emerging forensic potential of these technologies.

4. Infrared Spectroscopy (IR)

4.1. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is a fast, non-invasive, and highly effective analytical method that is routinely used for small molecule drug characterisation. The fundamental principle of FTIR spectroscopy involves the interaction of infrared radiation with a sample, where the absorption of light at various wavelengths is measured. This absorption occurs across three distinct spectral regions: near-infrared, mid-infrared, and far-infrared, each providing unique insights into the molecular structure of the sample, with the mid-IR region (approximately 4000–400 cm^{-1}) being the most widely used as it contains the fundamental vibrational modes of most functional groups in organic molecules. As the IR radiation passes through the sample, it generates an interference pattern, which is captured by a detector. This pattern, known as an interferogram, is then mathematically converted into a frequency-domain spectrum using a Fourier transform. The resulting spectrum illustrates the amount of infrared light absorbed or transmitted at different frequencies, revealing the unique vibrational signatures of the sample's molecular components [42]. FTIR has been extensively used for the screening and analysis of small molecule counterfeits. Numerous studies have successfully used FTIR, both independently and in combination with multivariate analysis, to distinguish between genuine and counterfeit tablets of small molecule medications and to analyse simulated counterfeit formulations. Beyond tablet analysis, FTIR has proven valuable in identifying unique spectral profiles within counterfeit batches, as well as in examining packaging materials such as inks, papers, and plastic components of blister packs and vials, to detect inconsistencies indicative of falsification. These applications highlight FTIR's versatility not only in chemical profiling but also in forensic packaging analysis, making it a critical tool in the ongoing fight against counterfeit drugs [43].

Proteins also absorb infrared light at specific frequencies corresponding to the vibrational modes of their chemical bonds, particularly those in the peptide backbone. There are various characteristic FTIR absorption bands for proteins, with the most informative region being the Amide I band (~1700–1600 cm^{-1}). This band arises mainly from the C=O stretching vibrations of the peptide bond and is highly sensitive to the protein's secondary structure, providing information related to the presence of α-helices, β-sheets, and random coils, as illustrated by Arunkumar et al. [44]. In their study, the authors demonstrated that shifts and changes in peak shape in the Amide I region of α-amylase reflect

loss of α-helical content and increased β-sheet formation in the presence of high concentrations of protic ionic liquids (Figure S1). Other regions such as the Amide II band (~1600–1500 cm^{-1}) associated primarily with N-H bending vibrations, or the Amide III band (~1400–1200 cm^{-1}) originating from a combination of N-H bending and the C-N stretching vibrations, even though do not provide detailed information on secondary information compared to the Amide I region, they offer valuable complementary structural information [45–48].

FTIR is often used as a complementary analytical tool in biopharmaceutical characterisation for the analysis of secondary structure of protein drugs, offering valuable insights into their aggregation state, folding behavior, and dynamics, as well as the presence of posttranslational modifications [49]. However, its use in forensic protein based drug detection has not been extensively investigated. Counterfeit biopharmaceutical products containing reduced concentrations of API typically exhibit diminished peak intensities in the FTIR spectrum at regions corresponding to the API. In cases where the API is entirely absent, the resulting spectrum closely resembles that of the formulation buffer. Furthermore, if an incorrect or adulterated substance is present- particularly one that alters the protein's secondary structure- these structural deviations will manifest as distinct differences in the FTIR spectral profile, especially within the amide I and II regions.

Godzo et al., [50] explored the potential use of FTIR with attenuated total reflectance (ATR) for the identification of trastuzumab in biopharmaceutical preparations. Researchers prefer to use ATR to avoid the complexities associated with water absorption signals that hinder spectra interpretation, however, a study explored the transmission mode for protein identification. When they analysed a protein drug developed for the treatment of malignant diseases, they were able to obtain information on the identity, secondary structure, purity, and structure effects resulting from pH variations, phosphate binding, and aggregation [51]. A review by Yang et al. [52] summarises recent progress in FTIR protein analysis that involves the utilisation of micro cell sampling methods to overcome water absorption band overlap in the Amide I region or using solid-film sampling as an alternative to the more complex micro cell solution sampling. This latter approach has proven to be effective at lower protein concentrations, making it comparable to CD and fluorescence spectroscopy in terms of sample requirements. As a result, solid-film FTIR sampling broadens the accessibility and applicability of FTIR in protein structural studies. In addition, the use of chemometrics further broadens the potential applications of FTIR. ATR mode and a partial least squares (PLS)-based chemometric model enabled the creation of a library of signature spectra that allowed subsequent automatic identification of protein drugs in comparison samples, as well as the calculation of its concentration without losing sensitivity or robustness [53]. The authors state that this method can be used as a "go-to" method and a valuable tool for rapid screening of potential counterfeits. Beyond conventional FTIR, advanced infrared modalities such as two-dimensional infrared (2D-IR) spectroscopy offer deeper structural insights.

2D-IR spectroscopy introduces a second frequency dimension to the IR spectrum, allowing the resolution of overlapping peaks and reducing spectral congestion as well as revealing couplings between vibrational modes, which are crucial for understanding secondary structure elements, thus making it suitable for large molecule analysis. This allows researchers to observe real-time conformational changes, hydrogen bond dynamics, and solvent interactions, which are not accessible with FTIR. Furthermore, when combined with isotope labeling, 2D-IR can selectively probe individual residues or segments of a protein, offering site-specific structural insights [54]. 2D-IR spectra offer richer and more detailed information on protein structure than traditional linear FTIR techniques, however, they pose significant challenges for use in forensic analysis. 2D-IR protein data remains complex to interpret, requiring specialised knowledge and equipment, which may not be practical in routine forensic labs. It also requires highly pure, concentrated samples, which may not be feasible with degraded or trace forensic evidence. Furthermore, the instruments themselves are expensive and not widely available, further limiting their use in standard forensic workflows. However, significant advancements over recent years in ultrafast laser technology, improved detector sensitivity, and faster data processing by the incorporation of statistical analysis and machine learning [55] hold significant potential for future forensic applications involving protein based drugs.

4.2. Near Infrared (NIR) Spectroscopy

NIR spectroscopy lies in the absorption of near-infrared light by molecular overtones and combinations of fundamental vibrations, primarily involving C–H, O–H, and N–H bonds, and is routinely used for small molecule pharmaceutical analysis [56]. NIR has also been proven to be an indispensable tool for counterfeit drug detection as its fast, non-destructive, and sample preparation-free requirements make it an ideal tool for forensic field testing using portable devices [57]. Portable NIR looks promising for biological sample analysis in forensic investigations, as

demonstrated in a recent study that evaluated the effectiveness of handheld NIR spectroscopy as a non-destructive, rapid, and portable tool for the forensic identification of body fluids. The researchers tested the ability of a handheld NIR device to distinguish between various biological fluids, such as blood, saliva, semen, and urine, on different substrates and under varying environmental conditions. Their findings demonstrated that NIR spectroscopy, when combined with chemometric models, could reliably differentiate between body fluids with high accuracy, even after exposure to environmental degradation [58]. However, when it comes to large molecule characterisation, as in the case of protein drugs, NIR spectroscopy has significant limitations. While it is highly effective for detecting broad chemical features such as moisture content, concentration, and general compositional changes, making it suitable for process analytical technology (PAT) applications in biopharmaceutical manufacturing [59], it lacks the spectral resolution and sensitivity required to probe the secondary and tertiary structures of proteins. NIR spectra are dominated by broad, overlapping overtone and combination bands, which obscure the fine structural details necessary for distinguishing between different protein structural characteristics. As a result, NIR is currently limited to the quantitative monitoring of protein concentration or formulation attributes rather than structural characterisation. One notable study, however, involved the use of a portable handheld NIR spectrometer to collect spectra from authentic and counterfeit samples of a lyophilised biologic product straight from the glass vials. The researchers demonstrated that NIR spectroscopy, when paired with chemometric analysis, could reliably identify differences in the chemical composition and formulation of the samples. This approach allowed for the generation of a spectral fingerprint of the authentic product, which could then be used for the rapid screening of counterfeits [60].

4.3. Microfluidic Modulation Spectroscopy (MMS)

One rapidly emerging IR technology for protein structural characterisation that could be applied to forensic detection is microfluidic modulation spectroscopy. Microfluidic modulation spectroscopy (MMS) is a novel automated mid-IR spectroscopic technique that can be used for the characterisation of protein secondary structure. Absolute absorbance and second derivative measurements can provide information on structural differences that take place across the secondary structure of proteins, such as beta sheets, alpha helices, or unordered regions. MMS works by flowing both the protein sample and a matching buffer reference through a microfluidic transmission cell. These two streams are rapidly alternated across the path of a tunable quantum cascade laser, which emits mid-infrared light, providing very high sensitivity that enables the detection of small changes in structure over wide concentration ranges with LOQs significantly lower compared to conventional FTIR [61]. Liu et al. [62] demonstrated that MMS can be used for the secondary structure analysis of mAbs, producing results comparable to an FTIR method, with the added advantages of higher sensitivity, repeatability, and automated operation. A high level of sensitivity able to detect subtle protein structural changes was also achieved in a recent study by Yang et al. [63], where nine mAbs were tested under different formulation buffers, yielding highly reproducible results. MMS is gaining rapid pace in biopharmaceutical development thanks to its high automation, wide dynamic range, broad buffer compatibility, and ease of use, which allow for the rapid fingerprint generation of protein higher order structure. These attributes make it a very promising IR technology that could find applications beyond biopharmaceutical development and into the forensic laboratory. While IR-based techniques offer valuable insights into protein structure, Raman spectroscopy provides complementary molecular information that holds increasing promise in counterfeit biologic detection.

4.4. Raman Spectroscopy

Whereas IR spectroscopy measures the absorption of infrared light by molecules which causes changes in their dipole moment during vibration, Raman spectroscopy measures the inelastic scattering of monochromatic light. As the light photons collide with molecules, most are elastically scattered (Rayleigh scattering), but a small fraction undergoes energy shifts due to vibrational energy changes in the molecules—this is known as Raman scattering. These energy shifts provide a molecular fingerprint unique to the chemical structure and environment of the sample [64,65]. Raman spectra, typically collected in the 10–4000 cm^{-1} range, are particularly useful due to their sharp peaks and sensitivity to non-polar functional groups, such as C=C and C=N. Raman spectroscopy has been routinely used for the analysis of small molecule pharmaceuticals since the late 90's playing a crucial role throughout the drug development lifecycle. Its applications include preformulation screening and the prediction of pharmacokinetic behavior, providing information on solid-state properties and drug-excipient interactions. Additionally, Raman spectroscopy is instrumental in the characterisation of drug delivery systems, offering insights into formulation stability and performance. Furthermore, as a robust Process Analytical Technology (PAT) tool, it enables real-time, non-destructive monitoring of manufacturing

processes. In addition, its utility in quality control further supports the identification of polymorphs, detection of contaminants, and verification of product integrity, making it an indispensable technique in modern pharmaceutical development and production [66].

Numerous studies have demonstrated the effectiveness of Raman spectroscopy in detecting counterfeit small molecule pharmaceutical products by analysing both APIs and excipients. Researchers have demonstrated that Raman spectroscopy can identify counterfeit drugs by detecting differences in excipients or polymorphs. Investigations using benchtop Raman spectrometers and microscopes have successfully distinguished authentic from counterfeit tablets of drugs like Viagra, Cialis, and Levitra. Raman spectroscopy has also been applied to packaging using spatially offset techniques. Advanced methods like Raman microspectroscopic mapping allow for spatial resolution of ingredients within tablets, enabling detailed comparison of authentic and counterfeit products based on ingredient distribution and spectral profiles [67].

When it comes to biopharmaceutical development, Raman spectroscopy plays a crucial role in the development and quality control of protein-based biopharmaceuticals due to its ability to provide rapid, detailed molecular information in aqueous environments in a non-destructive manner. The signals derived from proteins using Raman spectroscopy can provide complementary information to IR data (Table S1) and mainly come from two sources: the backbone of the protein chain and the side groups of the 20 different amino acids. In the lower energy range (called the fingerprint region), a typical protein shows around 30 distinct signals. There are also a few signals in a higher energy range that come from specific chemical groups such as NH, OH, CH_3, CH_2, and SH. These groups can form hydrogen bonds, which can change the shape of their signals depending on how they're interacting with their surroundings. Backbone signals can be used to reveal information on the protein's overall shape, while the side group signals help reveal details about the local environment, such as hydrogen bonding [68–70]. Raman can provide information on the secondary and tertiary structure of the API, able to detect post translational modifications and changes resulting from aggregation or after forced degradation studies [71–73], on particle formation [74], as well as on excipient concentration and information on PQAs (Product Quality Attributes) such as pH and osmolarity [75]. Hence, Raman spectroscopy is ideally suited for PAT (Process Analytical Technology) frameworks that can monitor critical quality attributes in real time. Its compatibility with aqueous environments and minimal sample preparation make it especially suitable for in-line or at-line monitoring in bioprocessing.

Raman's ability to generate distinct spectral fingerprints of protein-based therapeutics (Figure S2) without the need for extensive sample preparation makes it a compelling candidate for rapid screening in both field and laboratory settings for forensic investigations. Recent studies have begun to demonstrate the practical feasibility of Raman-based approaches for identifying counterfeit biologics, offering insights into both structural integrity and compositional anomalies. The following examples illustrate how Raman spectroscopy has been applied to distinguish authentic biologic products from their counterfeit counterparts.

A paper from a manufacturer described the use of an FT-benchtop Raman instrument operating with a laser source at 1064 nm used to analyse both authentic and counterfeit biologic drug samples directly through their glass vials, without any sample preparation. The biologics, presented as lyophilised cakes, appeared visually identical, and due to their amorphous nature, exhibited broad Raman peaks rather than the sharp peaks typical of crystalline substances. Despite the relatively low spectral resolution, the combination of high laser power and multiple scans produced spectra with a good signal-to-noise ratio, allowing for the identification of distinct features in the fingerprint region. The key spectral difference between the authentic and counterfeit samples was the absence of the amide I peak at 1672 cm^{-1} in the counterfeit, a peak associated with the carbonyl vibration in protein secondary structures and commonly used to detect the presence of active protein-based drug components. This absence, corroborated by subsequent capillary electrophoresis results showing no major protein peak, indicated that the counterfeit lacked the active ingredient and was likely a placebo. Although the counterfeit spectrum closely resembled the authentic one aside from the missing amide I peak and an unassigned shoulder at 3050 cm^{-1}, the findings emphasised that Raman spectroscopy can confirm the presence of protein but not necessarily verify the authenticity of the biologic. The study concluded that the counterfeit sample did not contain any active biological protein, and subsequent NIR spectroscopy results further supported this conclusion. [60].

In another study, researchers used Raman microscopy and the Drop Coat Deposition Raman (DCDR) technique to create spectral fingerprints for three different proteins with significantly different secondary structures, Bovine Serum Albumin (BSA), Ovalbumin, and Immunoglobulin G (IgG). By creating Raman spectral fingerprints of model proteins, the team demonstrated that each biologic has a unique vibrational signature. These signatures can be used to verify the authenticity of

a sample in counterfeit detection studies. The DCDR method was especially effective because it required minimal sample preparation, used very small sample volumes, and avoided the need for complex spectral corrections [76].

Another research effort examined twelve different drugs, in both liquid and lyophilised forms, directly through their glass packaging using Raman spectroscopy, and ten were further examined using Raman microscopy. The researchers optimised data acquisition settings, identified characteristic protein spectral bands, and successfully applied these methods to detect seven counterfeit samples. These counterfeits were identified based on differences in excipient profiles, absence of protein, or significant dilution of the original product. Overall, the study confirms that Raman-based techniques are efficient and reliable for the fast screening of counterfeit biologic drugs. However, the authors emphasised that acquiring Raman spectra from biologic samples, particularly those with low concentrations, poses greater challenges than analysing solid forms. Several technical factors significantly influence the quality of the spectra. Among them, the laser wavelength is critical- protein bands were more clearly visible when using a 532 nm laser. However, this wavelength also introduced more fluorescence, necessitating spectral correction. The microscopic method enabled the observation of more protein bands, thus potentially being a more suitable method for less concentrated products [77].

The benefits of a 532 nm laser for protein analysis were also observed in a more recent study featuring a handheld Raman device. The 532 nm laser excitation offers, according to the manufacturer, enhanced signal strength, making it suitable for the detection of counterfeit biologic drugs. The device enables analysis through glass or plastic containers without requiring direct contact with the sample. In a white paper published in 2023, Mikhonin and Hodi simulated counterfeit versions of erythropoietin and Avastin by using diluted API samples or omitting the API entirely. Their findings demonstrated that the 532 nm handheld Raman device effectively identified these counterfeit biologics [78].

4.5. Combined Approaches

In recent years, IR and Raman spectroscopy have increasingly been combined with other analytical technologies to enhance their capabilities for pharmaceutical applications. These hyphenated techniques, including methods such as AFM-IR (Atomic Force Microscopy–Infrared Spectroscopy), allow researchers to obtain both chemical and spatial information at micro- to nanoscale resolution. This integration has proven especially valuable for studying protein structures, drug formulations, and protein aggregation with a level of detail that traditional IR spectroscopy alone cannot achieve [79]. Furthermore, Raman imaging techniques can be used to visualise the distribution of proteins and excipients during phase separation in lyophilised products [80]. Raman microscopy has also been employed in several studies for counterfeit screening, as discussed earlier. However, while these advanced techniques offer powerful insights, they also introduce significant complexity in terms of instrumentation, data interpretation, and operational expertise. This makes them less practical for routine forensic detection and analysis of protein-based drugs, where simplicity, speed, and standardisation are often critical. Nevertheless, these technologies are evolving rapidly, and recent developments- such as improved automation, miniaturisation, and user-friendly software [81–84] could make them more accessible and practical. As such, they represent a promising frontier for forensic analysis and are well worth monitoring for their potential to transform how biological drugs are analysed and authenticated in forensic investigations.

Furthermore, integration of multiple spectroscopic techniques enhances the classification accuracy and predictive strength of analytical models compared to those relying on a single spectral method. While hyphenated techniques integrate spatial and chemical analysis at the micro- to nanoscale, multimodal approaches combine spectral data from distinct modalities to improve classification accuracy and robustness. Studies have demonstrated that combining spectral data can significantly improve the ability to distinguish between genuine and counterfeit products for small molecule drugs such as those used for erectile dysfunction and as muscle relaxants [85,86]. While these techniques have been predominantly applied to small-molecule drugs, the same principles can be extended to protein-based therapeutics. Provided that suitable reference spectra are available, combining Raman and IR spectral data could offer a powerful tool for detecting falsified or substandard protein medicines. This is particularly relevant given the structural complexity and sensitivity of protein drugs, where subtle changes in formulation or degradation may not be evident solely through packaging inspection. Thus, integrating spectroscopic fingerprints from multiple modalities holds promise not only for small molecules but also for safeguarding the integrity of biologics.

5. **Advancing Forensic Detection of Counterfeit Biologics**

The detection of counterfeit biologic drugs remains a significant global challenge, particularly in low-resource settings where infrastructure, expertise, and access to advanced analytical tools are limited. A recent WHO report

describing the analytical technologies used to screen and detect substandard and falsified drugs reveals significant gaps in the available evidence needed to guide policy decisions on selecting the most appropriate technologies for screening medicines suspected of being substandard or falsified, including when and how these technologies should be applied before confirmatory testing. The report also discusses the numerous obstacles countries face in setting up systems to detect falsified and substandard medicines, the most prevalent being a lack of technical knowledge. The report highlights specific challenges in testing complex products, such as biologics and vaccines, noting that many countries lack the necessary infrastructure, equipment, and trained personnel to carry out effective detection [87]. These limitations call for a more strategic and informed approach to selecting analytical tools that are not only scientifically robust but also practical and scalable.

Biopharmaceutical analysis is inherently designed to assess the identity, purity, and structural integrity of biologic drugs; parameters that are directly relevant to counterfeit detection. Vibrational spectroscopy tools, such as Raman and IR spectroscopy, are already being validated and standardised in the biopharmaceutical industry for quality control and regulatory compliance. This can make them more readily transferable to forensic applications focused on counterfeit detection.

Furthermore, it is important to remember that forensic drug testing is conducted both in the field and in laboratories. An ideal counterfeit biologic drug screening tool should be portable, resistant to environmental elements, able to perform fast and reliable measurements, generating actionable results for non-expert users, while adhering to regulatory standards. Although there are still limitations on data handling, standardisation, and quantitative analysis by current IR and Raman portable instruments [88], simplicity in operation minimises training needs and affordability, and the potential for future cost reductions will be critical for widespread adoption [67]. Although the use of Raman and IR spectroscopic technologies in detecting counterfeit biological drugs is currently in its infancy, ongoing advancements in instrumentation and data analysis are expected to overcome existing barriers and offer fast, non-destructive, and highly reliable detection of counterfeit biologics, even outside laboratory environments.

Building on this, it is crucial for forensic science to actively monitor and evaluate the evolving usability of Raman and IR spectroscopy in the biopharmaceutical industry, just as these technologies are being integrated into biopharmaceutical development. The forensic field must not only stay informed but also be proactive in adopting new methodologies that enhance the detection and rapid characterisation of complex biological drugs. Even though the focus of this study is on protein and peptide-based drugs, emerging therapies such as gene therapies, cell-based treatments, and other complex biologic products present growing challenges for counterfeit detection. These advanced therapies often involve intricate molecular structures and highly specific manufacturing processes, making them both valuable and vulnerable to counterfeiting. As the biopharmaceutical landscape continues to evolve, so must the tools used to protect it. Forensic science can benefit from these advancements by adapting them to its own unique challenges.

6. Conclusions

The growing threat of counterfeit biologic drugs demands innovative, interdisciplinary solutions. Current analytical workflows are complex, requiring advanced technical expertise, and are largely non-field deployable. In addition, many regions face infrastructural and technical barriers to implementing advanced detection methods. There is a pressing need for validated spectroscopic tools that can be deployed in both field and laboratory settings. Such tools would significantly enhance the ability to detect counterfeit biologic drugs in real-time, especially in low-resource environments where timely decision-making is critical. As this paper has demonstrated, vibrational spectroscopy, particularly IR and Raman technology, presents a promising approach for the rapid, non-destructive authentication of protein- and peptide-based therapeutics. These technologies, already well-established in biopharmaceutical development, could become increasingly adaptable to forensic workflows, especially with advancements in portability, automation, and data analysis. Interdisciplinary collaboration between forensic scientists and biopharmaceutical developers is essential to ensure that emerging technologies are effectively translated into practical solutions. By fostering collaboration across disciplines, forensic science will ensure that, as counterfeiters become more sophisticated, the tools used to detect them remain one step ahead.

Supplementary Materials

The following supporting information can be found at: https://www.sciepublish.com/article/pii/765, Figure S1: Amide I region of FTIR spectra of α-amylase in aqueous solutions of 0, 5, 10 and 20 mol% of ethylammonium nitrate, showing progressive changes in secondary structure. Reproduced from [44], under Creative Commons Attribution License (CC BY). Copyright © 2019 Arunkumar, Drummond and Greaves; Figure S2: Raman spectra of 15 therapeutic

monoclonal antibodies, with the characteristic spectral region highlighted by a red dashed box. Reproduced from [89] under Creative Commons Attribution License (CC BY). Copyright © 2022 Ling, Zheng, Xu, Chen, Wang, Mao and Shao; Table S1: Commonly used IR and Raman spectroscopy spectral regions for protein structure analysis [45–48,68–70].

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the author(s) used an AI language model (Microsoft Copilot) in order to improve grammar, syntax and clarity. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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The author declares that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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