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Body Fluids and Spectroscopic Techniques in Forensics: A Perfect Match?

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Abstract

Human body fluids are of great interest in forensics, due to the possibility to extract their genetic information. At the moment, there is the need to develop a non-destructive, rapid and user-friendly method for the detection and identification of the body fluids usually found at crime scenes: blood, semen, vaginal fluid, saliva, sweat and urine. In this review, the spectroscopic techniques used or being researched on this topic are discussed, taking into account their advantages, limitations and advances. Although, UV-Vis light sources are used worldwide in forensic laboratories for the location of body fluids, they are not selective enough to be confirmatory. HSI, FTIR and Raman spectroscopy seem to be suitable for the identification and discrimination of body fluids, though comprehensive research about some unsolved aspects must be performed first.

Keywords: Blood; Body fluids; HSI; IR; Raman spectroscopy; Saliva; Semen; UV-Vis; Vaginal fluid

Introduction

Biological evidence has become extremely decisive in forensics. Their presence within the crime scene usually provides a great deal of useful and reliable information to forensic practitioners concerning not only details about the causes and consequences around the evidence, but also knowledge regarding the identity of their owners. This valuable information is obtained through the genetic identification of the DNA (deoxyribonucleic acid) contained in the evidence. The term biological evidence includes a wide variety of remains such as bones, hair, skin and blood, amongst others. Particularly, blood and other body fluids including semen and saliva are extremely useful for the investigation of many crimes related to sexual abuse cases, assault and battery crimes and homicides. Actually, these three body fluids (especially blood) have been studied in greater detail than others over the years because of their constant and noticeable presence in both crime scene and victim [1-4]. However, it is not always easy to confirm their presence, in fact, many times it is certainly challenging to detect and identify remains of body fluids.

Different presumptive and confirmatory tests are available and are currently being used to locate, identify and confirm these body fluids [2]. These two categories of tests (presumptive and confirmatory) are distinguished by their specificity and, therefore, the confidence level in the identification of the body fluid. Confirmatory tests allow the unequivocal identification of body fluids whereas presumptive tests only provide a preliminary sign of the presence of the body fluid. Table 1 reports a summary of the most relevant presumptive and confirmatory tests currently used to identify blood, semen, saliva, vaginal fluid, urine and sweat. Afterwards, this review reveals some discouraging troubles when using these tests such as the fact that each body fluid requires its specific tests [1,2] and, furthermore, the fact that confirmatory tests, which only exist for blood and semen, are destructive [1,5]. These unfavourable drawbacks and limitations make definitely necessary the exploration of additional analytical techniques in order to develop a novel methodology: a definitive, unique and non-destructive test that can be suitable for the identification of every body fluid. Although this ambitious and challenging purpose may seem impossible to achieve, the reality is that there is not one approach, but two approaches; one from genetics and the other from analytical spectroscopy [1].

The genetic approach involves the development of specific mRNA (messenger ribonucleic acids) markers for each body fluid. It is widely known that every cell from an individual has in common the same DNA within its nucleus. On the contrary, not every cell shares the same proteins [6]. That undeniably means that mRNA, which is translated into proteins, is different from cell to cell. Since body fluids include some cells in their composition, either from themselves (e.g. leucocytes - blood, spermatozoa - semen) or epithelial cells from the body parts with which they keep contact (e.g. buccal, bladder, prostate and vaginal epithelial cells), the identification of body fluids based on their different mRNA sequences is a feasible approach [7].

Moreover, the rapid advance developed for DNA analysis lately can be easily applicable to RNA analysis including amplification by PCR and sequencing of nucleotides. Nevertheless, despite this promising approach for the unequivocal confirmation of body fluids, it displays some limitations for practice detection and location of body fluids. In fact, these are the same limitations that are characteristic of DNA analysis such as being very expensive and slow. This mRNA approach is undoubtedly perfect to confirm the body fluid when the sample has been collected. However, it is necessary first a method to identify the body fluid prior to RNA and DNA analysis.

The approach from analytical spectroscopy seems more suitable. This strategy consists of identifying the body fluids based on their characteristic spectral signatures. Spectroscopic techniques present attractive features such as being fast, solvents-free, cost-effective, easy-to-use and even portable to work on-field [1].

Type of test	Blood [1-4]	Semen [1-4]	Saliva [1-4]	Vaginal fluid [1, 2]	Urine [1. 2]	Sweat [1, 2]
Chemical	Presumptive: Luminol Kastle-Meyer Leucomalachite green Benzidine Fluorescein	Presumptive: Acid phosphatase Choline	Presumptive: Amylase	Presumptive: Periodic acid Schiff	Presumptive: Urea Jaffe Salkowski Ureic acid/ urea ratio	N/A
Spectroscopic	Presumptive: ALS Confirmatory: UV-Vis (Soret band)	Presumptive: ALS SEM-EDX	Presumptive: ALS SEM-EDX	N/A	Presumptive: - ALS - SEM-EDX	Presumptive: SEM-EDX
Microscopic	Confirmatory: Microscopic visualization	Confirmatory: Christmas tree	N/A	N/A	N/A	N/A
Immunological	Confirmatory: Antibodies	Confirmatory: PSA or PS30 Other ab	N/A	Presumptive: Estrogen receptors	Presumptive: Tamm-Horsfall (THP)	N/A
Electrophoretic	N/A	N/A	N/A	Presumptive: Vaginal peptidase Lactate/ citrate ratio	N/A	N/A
Crystal test	Confirmatory: Takayama crystals Teichman crystals	Presumptive: Florence Barberio	N/A	N/A	N/A	N/A
Chromatographic	N/A	Presumptive: Spermine	N/A	N/A	Presumptive: 17-Ketosteroid	N/A

Table 1: Compilation of assays for body fluid detection. Adapted with permission from [1].

In fact, spectroscopy is already being used for the presumptive detection and location of body fluids by means of forensic light sources that work along visible and ultraviolet (UV-Vis) range. In addition, the application of more selective techniques, including near infrared hyperspectral imaging (NIR-HSI), Fourier transform infrared (FTIR) and Raman spectroscopy, is being investigated. The most relevant advances focused on identifying body fluids using these three spectroscopic techniques are discussed below.

Body fluids and spectroscopic techniques in forensics

Current role of UV-Vis spectroscopy for body fluids stains detection

Most body fluids undergo fluorescence or absorption processes when they are irradiated by UV waves. This fact allows their rapid detection over small but also wide areas by using forensic lights. In summary, since 1987 several authors have compared different UV-Vis lamps for body fluids detection. For instance, Auvdel [8] compared the detection of saliva, semen and sweat stains, at different concentrations, on several clothes by using different UV-Vis lamps. However, results were not satisfactory because of the short UV-Vis range available within the UV-Vis lamps used. In the following year, the same author tested an UV-Vis lamp working along a wider wavelength range, and better results were achieved due to the increase of stains fluorescence [9]. By using a wider range of UV-Vis wavelengths, Stoilovic, in 1991, achieved the detection of dried semen and blood stains on photo luminescent and non-photo luminescent substrates [10]. In addition, some procedures for the correct detection of semen and blood stains were defined from this work. Subsequent studies by using the same or similar UV-Vis lamps have been published by different authors focusing on the detection of semen, blood, saliva and urine stains on several white and coloured fabrics including cotton, polyester, nylon and wool [11-16]. Interestingly, the optimum UV-Vis excitation wavelengths for each body fluid (skin oil, semen, blood, urine and saliva) have been reported, proving that semen and urine seem to have a wider range of wavelengths in which they can be detected [17]. Since then, UV-Vis lamps have been used in forensic laboratories worldwide for detecting stains of body fluids on different substrates.

However, according to those studies, the selectivity provided by UV-Vis spectroscopy range is quite poor since the body fluids have a similar response within its range. Consequently, this technique does not enable the identification of body fluids [1]. It also displays other limitations in the detection of body fluids, including the existence of several substances that respond as false positives and the interference of coloured backgrounds from fabrics and other materials, on which the stains are deposited, which may hinder the identification of body fluids. Therefore, UV-Vis spectroscopy cannot be used beyond exploratory and presumptive identification purposes. Nonetheless, UV-Vis spectroscopy is noticeably satisfactory to preliminary locate

stains of body fluids along wide areas; stains which will have to be subsequently confirmed by more selective techniques.

Vibrational spectroscopy in promising approaches for body fluids identification.

IR range is particularly suitable for the forensic identification of body fluids because IR wavelengths are correlated with the chemical vibrations in molecules, which establish the characteristic bands observed in IR or Raman spectra. Nowadays, both IR and Raman spectroscopy are being investigated for the identification of body fluids and promising results are being obtained. Concerning IR spectroscopy, both NIR and mid-IR (MIR) ranges are being explored and different instrumentation including hyperspectral imaging (HSI), transmittance, diffuse reflectance or attenuated total reflectance are being investigated.

HSI, as well as other imaging techniques such as MIR thermal multimode imaging, seems to be highly suitable to detect stains of body fluids since it combines the features from both photography and IR spectroscopy. In fact, this combination provides both spatial and spectral information of samples enabling the visualization of stains based on their characteristic spectra. However, most of the studies are exclusively focused on blood detection [18-23]. For instance, Morgan et al. [18, 19] identified diluted blood stains on dark fabrics made of acrylic fibre, cotton, nylon and polyester through the contrast between blood and background when using MIR thermal imaging. They also demonstrated that rust, cherry soda and coffee, known to be possible false-positive interferences, did not provide misleading signals equivalent to blood. In a similar way but using NIR-HSI instead of thermal MIR, Lin et al. [20] demonstrated the advantages of NIR over Vis range by analysing diluted blood stains on black fabrics made of different materials including cotton, acrylic, polyester, wool, rayon, lycra and velvet. The characteristic spectral signature of blood along the NIR range (760-1500 nm) provided higher selectivity than using solely the Vis range (400-780 nm). In the same way, De Forest et al. [21] tested NIR (850 nm) versus a Vis wavelength range (400-700 nm) to identify blood stains on dark clothes made of cotton, polyester, wool, leather and silk. Interestingly, they studied four types of blood stains (i.e. smear, contact, drop and small spatter) usually found in real crime scenes. De Forest et al. [21] also examined potential false-positive substances including red inks, wine, ketchup and lipsticks among others. In brief, results again proved the superiority of NIR to detect blood stains except for the "small spatter" stains and most of the substances tested were easily distinguished from blood. Schuler et al [22] overcame these results using a wider NIR range (650-1100 nm and 960-1650 nm) and demonstrated the detection and visualization of both transfer/smear and spatter blood stains on black fabrics (cotton and denim). Furthermore, the discrimination among stains of blood and potential false-positive substances such as lotions and lipsticks was achieved based on their different NIR spectral response. A whole comparison between using NIR and UV spectroscopy versus the traditional chemical tests based on luminol, hydrogen peroxide and fluorescein reagents was performed by Finnis et al. [23] for the detection of diluted blood stains on dark fabrics made of cotton, polyester, wool, leather, suede, vinyl or rubber. Two types of blood stains were studied (drop and small spatter): "drop" stains were detected by using all methods whereas "small spatter" stains were only detected by chemical tests. In addition, the most diluted stains were only detected by using luminol and fluorescein tests. This fact seems to confirm that sensitivity of spectroscopic techniques is lower than those for chemical tests. However, this is understandable since spectroscopic responses are usually less intense than chemical reactions. It has to be assumed that the main advantages of IR spectroscopy include its selectivity, non-destructiveness, the speed of the analysis, the absence of solvents, reagents and any sample treatment and its capability to identify multiple body fluids (not only blood) and differentiate among them. Although NIR-HSI has not been examined for the detection of other body fluids besides blood yet, FTIR spectroscopy seems to demonstrate the identification of different body fluids as outlined below.

In fact, selectivity is increased when using MIR range and, fortunately, the research focused on discriminating among stains of different body fluids has been recently undertaken using MIR-FTIR spectroscopy. For instance, Elkins [24] studied the identification of blood, semen, saliva, vaginal fluid and urine stains on white cotton by ATR in the MIR region based on the characteristic spectral bands of each body fluid. In addition, other substances such as coffee, wine, chocolate, cheese, yogurt and lotions, amongst others, were tested as false positives. According to their different and characteristic spectra, the five body fluids were easily distinguished. Furthermore, they were clearly differentiated from the other substances. In the same way, Orphanou [25] focused on the discrimination between blood, saliva, semen and vaginal fluid by ATR-FTIR spectroscopy based on their different spectra. These spectra are shown in Figure 1. In addition, the main chemical components of body fluids were also analysed and those spectra were compared to body fluids. Briefly, human serum albumin and haemoglobin were tested for blood, α-amylase and lysozyme for saliva, acid phosphatase (AP), prostate specific antigen (PSA) and albumin for semen and AP, lysozyme and α-amylase for vaginal fluid. However, these spectra belong to the body fluids directly placed on to the ATR window and therefore, their identification on common substrates such as fabrics remained unsolved.

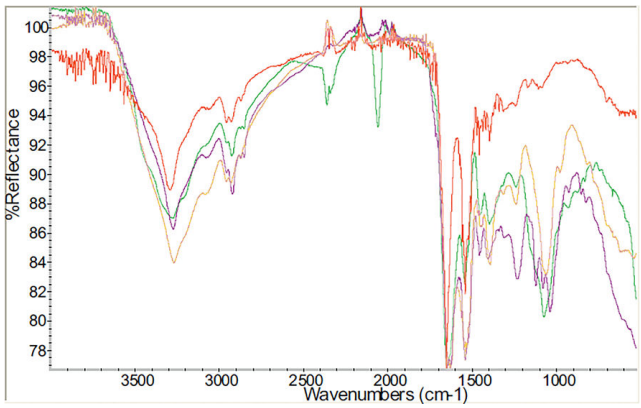

Figure 1: ATR FTIR spectra of blood (red), semen (orange), saliva (green) and vaginal secretions (purple). Adapted with permission from [25].

After revealing some disadvantages of ATR-FTIR spectroscopy for the analysis of body fluids stains on fabrics such as the need of pressing the fabric against the ATR window, the exploration of external reflectance FTIR spectroscopy for the identification and differentiation of semen, vaginal fluid and urine stains on white and coloured cotton fabrics was performed by Zapata et al. [26]. Significant differences within the spectra from the three body fluids allowed their identification and differentiation as shown in figure 2, which was

subsequently corroborated by chemometric methods such as Principal Component Analysis (PCA) and Soft Independent Modelling of Class Analogy (SIMCA). In addition, several substances including milk, yogurt, soap, sunscreen and juices, amongst others, were tested as false positives, but none of them was confused with a body fluid.

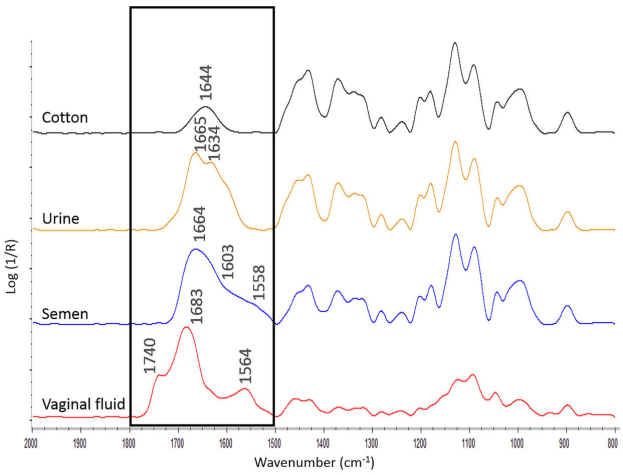

Figure 2: a) Reflectance FTIR spectra of cotton, and stains of urine, semen and vaginal fluid on cotton. Adapted with permission from [26].

With regards to Raman spectroscopy, there are also numerous studies that are exclusively focused on blood stains. A preliminary study proving the capability of Raman spectroscopy for the identification of blood stains by comparison with UV-Vis and ATR FTIR spectroscopy was fulfilled by De Wael et al. [27]. These blood stains which were made on fabric, glass and metallic surfaces came from three different species (human, dog and cat). Although all blood stains were detected by these three techniques based on their characteristic spectral signal, the extreme similarity among the blood spectra of the three species frustrated the aim of distinguishing them, either by UV-Vis, IR or Raman spectroscopy. This challenging purpose regarding blood species discrimination was subsequently tackled by Lednev's group, by using Raman spectroscopy followed by chemometric analysis [28,29]. In this respect, chemometric analysis such as PCA enabled the discrimination of blood species based on slight spectral differences, differences that are hardly visible or even unnoticed for the naked eye but highly significant by chemometric procedures [28]. Thereby, the differentiation of blood from several species (up to 12) including human, dog, cat, cow, horse, pig and chicken, amongst others, has been successfully achieved [29].

Some studies concerning the identification of diluted blood stains on different substrates by using normal Raman spectroscopy or surface enhanced Raman scattering (SERS) were performed by Boyd et al. [30,31]. The Raman signal due to blood was detected up to 1/250 diluted stains using normal Raman spectroscopy [30] and up to 1/105 dilution using SERS [31]. With regards to the substrates, non-luminescent substrates such as glass, plastic and wall provided the best results. The characteristic bands from blood were intense, well defined and no-overlapped. On the contrary, spectra from the blood stains on clothes were quite influenced by scattering bands from the fabric which reduced the intensity of the bands from blood and hindered their identification [30]. By using SERS, the influence of substrate signal and fluorescence, even in luminescent substrates such as fabrics, was removed [31].

The discrimination between different types of blood by Raman spectroscopy has been also studied by Lednev and his team for the purpose of discerning between menstrual and peripheral blood [32], information which can be relevant in many forensic cases. By using chemometric analysis, the differentiation between these two kinds of blood was achieved. In this case, it was explained that menstrual blood contains additional compounds from vaginal fluid besides the natural composition of peripheral blood, i.e. menstrual blood as a sort of mixture between peripheral blood and vaginal fluid [32].

The analysis of other body fluids besides blood using Raman spectroscopy has also been mainly researched by Lednev's group. In outline, the representative Raman spectra of blood, semen, vaginal fluid, saliva and sweat have been proposed as shown in Figure 3, information about their chemical composition has been obtained from the study of their spectral bands and several studies proving the differentiation of these five body fluids based on their characteristic Raman signatures by using chemometrics have been published [33-38]. Moreover, multivariate classification models have been developed in order to identify and classify unknown fluids, even in mixtures [39-41]. Nevertheless, the stains that were analysed in most of these studies were samples of body fluids on glass slides, not mimicking correctly real crime scene samples.

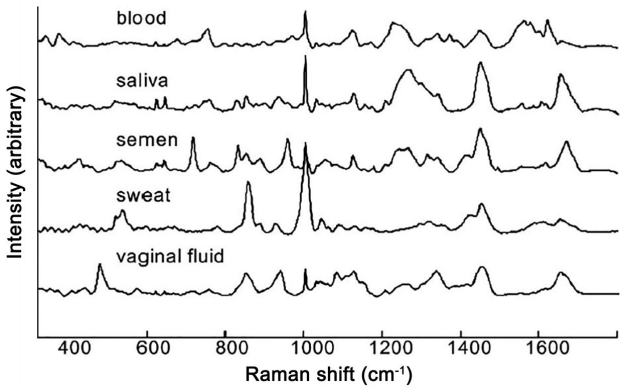

Figure 3: Raman spectra of blood, saliva, semen, sweat and vaginal fluid. Adapted with permission from [40].

Vibrational spectroscopy in forensics: present and future applications.

Vibrational spectroscopies as IR and Raman spectroscopy seem to overcome the low specificity of UV-Vis. The reviewed studies confirm that IR and Raman spectroscopy are highly suitable for the discrimination among body fluids. In addition no false positive substances have been found which may be erroneously identified as body fluids by these techniques [18,19,21,24,26]. Both IR and Raman spectroscopy are very selective, rapid, non-destructive and all the other features previously assessed for spectroscopic techniques. However, they are not routinely used by forensic practitioners yet.

This fact may be related to the need of specifying clearly the field of action of IR and Raman spectroscopy. In fact, it must be established whether IR and Raman spectroscopy are suitable for stand-off

detection of stains of body fluids during crime scene investigation like UV-Vis light sources, if they are suitable for confirmatory analysis within the laboratory, or both. In fact, HSI, FTIR and Raman spectroscopy offer different advantages and disadvantages for each purpose.

Portable hyperspectral cameras working along NIR range have been successfully tested for the detection of blood stains [20-23]. NIR range is less specific than MIR, but it is selective enough to preliminary identify blood stains. Assuming this, NIR-HSI is promising for the identification of evidence in crime scene including stains of body fluids. However, further research focused on other body fluids is necessary to clearly demonstrate its suitability.

Both IR and Raman spectroscopy seems to be particularly suitable for laboratory analysis to confirm the body fluid presence in a stain through its characteristic IR or Raman spectrum. Undoubtedly, Raman spectroscopy allows the identification and discrimination among body fluids. However, it seems not so suitable for the identification of stains of body fluids when they are on fabrics [30]. According to some studies, luminescent and coloured backgrounds provide intense Raman signals, which may hinder the identification of the bands from the fluid. That is because, besides the fluorescence generated by some fabrics, dyes from fabrics are extremely active in Raman [30]. Regarding IR spectroscopy, the MIR range is the most appropriate for identification purposes because it is more informative and therefore provides higher selectivity and confidence to confirm a body fluid [1]. Both ATR and external reflectance modes, working in MIR range, have demonstrated their capabilities to identify and differentiate body fluids, while it is true that external reflectance is preferable to ATR for the analysis of stains, as stated above [24-26]. Nevertheless, both external reflectance and ATR are being preliminary investigated at the moment for the discrimination of stains of body fluids and it will take time until overwhelming results prove the superiority of these techniques.

With regards to stand-off detection of forensic evidence at crime scene, both HSI, FTIR and Raman portable instruments are available [20-22,42-44]. For now, portable and miniaturized instruments have been used for detection of drugs and explosives, thanks to the wide available library containing these compounds [42-44]. In fact, these portable instruments require a library containing spectra from standards to provide an accurate result of identification based on the automatic matching between the measured spectrum and the spectra within the library. However, current libraries lack of spectral information concerning body fluids. Body fluids are challenging forensic evidence because they do not appear as isolated particles like drugs and explosives, but forming stains on different substrates or even they are absorbed within fabrics which also contribute to the vibrational spectra. Moreover, these portable instruments usually offer less features, facilities and performance than laboratory instruments. Therefore, it must be completed first the research by using these techniques in the laboratory and then the research concerning their miniaturization and application into the crime scene.

Another reason why IR and Raman spectroscopy are not used by forensic practitioners is probably due to the fact that almost all research has been performed with simulated samples within laboratory conditions. For example, a large part of the studies were carried out by analysing samples of body fluids on glass slides or directly placed onto the instrument [25,33-41], which is an extremely unusual substrate in real cases. In addition, studies in which stains were prepared on different clothes such as cotton, polyester, acrylic or wool, exclusively correspond to blood [18-23,27,30,31] except for studies [24,26]. There are still some unsolved questions concerning the identification of stains of body fluids (particularly semen, vaginal fluid, saliva, urine and sweat) on fabrics, such as the influence of the fabric material and colour to the IR or Raman spectrum. When stains on fabrics are analysed by IR or Raman spectroscopy, the spectrum obtained is not only due to the stain, but a combination between stain and fabric. Therefore, it is certainly necessary to examine the spectrum and check the presence of the characteristic bands from the fluid among the bands from the fabric. In fact, the bands from the fabric usually dominate the spectrum, overlapping in some cases the bands from the body fluid. Consequently, a comprehensive study testing stains of blood, semen, vaginal fluid, saliva, urine and sweat on a wide variety of white and coloured clothes made of usual materials such as cotton, polyester, acrylic, wool, silk, velvet or leather would be extremely useful for both IR and Raman spectroscopy.

In addition, the simulated stains analysed in research studies only contain the body fluid under study over the substrate. However, samples from real cases usually contain a mixture of several body fluids. The trouble related to mixtures of body fluids is particularly challenging for sexual abuse and rape cases in which a mixture of vaginal fluid, semen and even blood on victim's underwear, usually hinders the identification of semen [45]. Moreover, besides the victim's underwear, sanitary towels and panty liners are usually recovered as evidence in these cases [46]. However, up to now, the identification of stains of body fluids on these materials has not been studied by using spectroscopic techniques. In fact, sanitary towels and panty liners are particularly challenging for the analysis of stains because they contain a high percentage of absorbent materials that absorb and retain body fluids inside and avoid to a considerable extent the formation of stains on the surface. Consequently, the identification of body fluids on these materials by IR and Raman spectroscopy gets more complicated and require novel approaches to be achieved.

Therefore, it is necessary to test samples more similar to the evidence from real cases to demonstrate the real capabilities of spectroscopic techniques to forensic practitioners. Otherwise, vibrational spectroscopy will not be used for the forensic identification of body fluids beyond the academic and research field.

Conclusions

In forensics, spectroscopic techniques are being researched for the detection and identification of human biological fluids, important evidence to understand a crime scene and try to identify the aggressors.

Despite being used in forensic laboratories worldwide for the location of body fluids, UV-Vis light sources present several limitations for identification purposes because the fluorescence undergone under UV light by body fluids is not specific for each body fluid. Therefore, this technique is exclusively used as a presumptive assay.

NIR-HSI partially overcomes the limitations of selectivity from UV-Vis through the wider and more informative spectral range of NIR. In addition, HSI combines this spectral information with spatial information from the photographed sample. FTIR and Raman spectroscopy seems to be also two promising techniques for the identification of body fluids, because of their higher selectivity, which is superior than UV-Vis and NIR-HSI. In fact, both techniques allow a specific characterization for each body fluid according to their unequivocal IR and Raman spectra, which are correlated to the specific

chemical composition of each body fluid. In addition, the analysis by using these techniques are fast, solvent-free, cost-effective and non-destructive, which is mandatory to subsequently achieve an efficient DNA analysis in the forensic study of an evidence.

However, further research and comprehensive studies focused on their optimization and validation for the identification of stains of body fluids are necessary to be used in real forensic cases. In this respect, the optimum instrumentation for each purpose either presumptive detection in the crime scene or confirmatory identification in the laboratory must be investigated in detail. In addition, the influence of a wide variety of factors related to stains of body fluids must be studied such as the type of substrate or fabric and the mixture of several body fluids.

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