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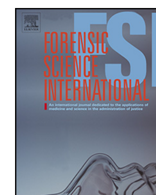

Rapid Communication

The detection and discrimination of human body fluids using ATR FT-IR spectroscopy

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A B S T R A C T

Blood, saliva, semen and vaginal secretions are the main human body fluids encountered at crime scenes. Currently presumptive tests are routinely utilised to indicate the presence of body fluids, although these are often subject to false positives and limited to particular body fluids. Over the last decade more sensitive and specific body fluid identification methods have been explored, such as mRNA analysis and proteomics, although these are not yet appropriate for routine application. This research investigated the application of ATR FT-IR spectroscopy for the detection and discrimination of human blood, saliva, semen and vaginal secretions. The results demonstrated that ATR FT-IR spectroscopy can detect and distinguish between these body fluids based on the unique spectral pattern, combination of peaks and peak frequencies corresponding to the macromolecule groups common within biological material. Comparisons with known abundant proteins relevant to each body fluid were also analysed to enable specific peaks to be attributed to the relevant protein components, which further reinforced the discrimination and identification of each body fluid. Overall, this preliminary research has demonstrated the potential for ATR FT-IR spectroscopy to be utilised in the routine confirmatory screening of biological evidence due to its quick and robust application within forensic science.

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1. Introduction

The UK National Crime Survey reported that over 1.3 million violent and sexual offences were recorded in 2013/14 [1]. At these types of crime scenes it would not be uncommon for human body fluids to be present, with the most frequently encountered body fluids being blood, saliva, semen and vaginal secretions which can play an important role within criminal investigations. The presence of these body fluids has significant forensic value as the identity of the person who deposited the body fluid can be determined with the use of deoxyribonucleic acid (DNA) profiling. DNA profiling has had a profound impact on the investigation of crime, allowing individuals of interest to be questioned, or potentially be eliminated as a suspect. However, a limitation of DNA profiling is that it cannot determine the source of the biological material.

When considering DNA as part of a criminal investigation, the source of the DNA can be as relevant to the context of the crime as the DNA profile itself. However, some sources of DNA at crime scenes may not be as obvious as others, especially when body fluid stains are present. Due to the variability in the visual appearance of body fluids some sources may not be easily identified. Blood has a characteristic red colour that can easily be distinguished from other body fluids. However, saliva, semen, and vaginal secretions can be much more difficult to see due to their creamy-white, or colourless appearance. An important aspect to consider is that stains present at a crime scene may appear similar in likeness to body fluid stains, but in fact be a stain from a non-biological source. To address this issue, the application of presumptive tests allows investigators to test suspect stains and fluids to detect the presence of particular body fluids [2]. There is an array of presumptive and confirmatory tests that have been used for many years to indicate or identify evidence containing biological material. Such tests include Kastle–Meyer (KM) and ABAcard® Hematrace® for blood [3], Phadebas® Forensic Press test for saliva [4] and AP and PSA, or p30, for the detection of semen [5,6]. To date there is no presumptive or confirmatory test available for vaginal secretions.

Abbreviations: AP, acid phosphatase; ATR FT-IR, attenuated total reflectance Fourier transform infrared spectroscopy; DNA, deoxyribonucleic acid; Hb, haemoglobin; HSA, human serum albumin; KM, Kastle–Meyer; mRNA, messenger ribonucleic acid; PSA, prostate specific antigen.

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As time and technology has progressed there has been little development of these traditional methods and as crime and criminals are becoming more intelligent, the techniques employed to identify body fluids need to become more sophisticated.

Within the last decade a plethora of research has been reported looking at new, innovative techniques to definitively identify body fluids. These techniques focus primarily on nucleic acid profiling of messenger ribonucleic acid (mRNA), micro RNA and methylated DNA [7–19], proteomics with mass spectrometry [20–22] and Raman spectroscopy [23–31]. Each of these techniques have demonstrated high specificity and sensitivity, although nucleic acid profiling and mass spectrometry techniques are still not appropriate for routine forensic application due to the time consuming and expensive methodologies and the lack of a standardised protocol. Raman spectroscopy has an advantage over nucleic acid and protein techniques due to the non-destructive, quick and easy application. This spectroscopic technique has demonstrated its successful application to definitively identify body fluids with the development of body fluid specific Raman spectroscopic signatures [23–31]. Within forensic science, vibrational spectroscopic techniques Raman and Fourier Transform infrared (FT-IR) are routinely utilised in the analysis of chemicals, drugs, fibres and paints [32–34], although their application to biological evidence is not commonplace. The development of Raman spectroscopic signatures of body fluids has demonstrated the potential for routine spectroscopic examination of biological samples.

Unlike Raman spectroscopy, FT-IR is routinely applied to biological samples within biomedicine for diagnostic purposes [35–39], although its application to samples of a biological nature has not been thoroughly explored within forensic science [40]. Attenuated total reflectance (ATR) FT-IR spectroscopy has been widely used within biomedical science to examine and identify biomolecule components and structures as all human tissues, cells and body fluids contain biomolecules which can be differentiated from one another by their unique structures [41,42]. FT-IR and ATR FT-IR spectroscopic techniques produce spectra containing bands, or peaks, representative of the vibrations of structural bonds and functional groups within biological samples. The positioning of the peaks are specific to particular interactions with molecular bonds and provide specific information relating to the biochemical composition [43].

Its application in biomedicine demonstrates that FT-IR spectroscopy is an ideal tool for the confirmatory screening of potential biological evidence prior to any costly and destructive DNA analysis. This would allow investigating officers to make informed decisions on any further analysis to be carried out on evidential samples, as well as providing an identification of the sample under examination, whether biological or chemical in nature. The aim of this research was to explore the use of ATR FT-IR spectroscopy as a non-destructive technique to successfully distinguish between different body fluids in order to determine body fluid stain source as a means of confirmatory identification.

2. Methodology

2.1. Instrumentation

All samples were analysed using the Nicolet 380 Fourier Transform Infrared (FT-IR) Spectrometer with a Smart Orbit, diamond crystal attenuated total reflectance (ATR) accessory and EZ Omnic (v7.4) software (Thermo Nicolet Corporation, United States of America). All samples were scanned 32 times per analysis at 4 cm^{-1} resolution within the 400–4000 cm^{-1} infrared region. The measurement format utilised was %Reflectance with data spacing at 1.929 cm^{-1}.

2.2. Sampling

Human blood, saliva, semen and vaginal secretions were the body fluids examined, with a total of 20 samples collected (five per body fluid type) and analysed at different time periods. Blood, saliva and vaginal secretions were collected from one donor, and semen was collected from a separate donor. Blood samples were collected using the finger prick method using an Accu-Chek Safe-T-Pro Plus lancet (Roche, Australia), saliva samples were collected in sterile, screw-top tubes via "spitting". Semen samples were collected by self-masturbation in to sterile, screw-top tubes. Vaginal secretion samples were collected using a Softcup [44]. All body fluid samples were collected fresh and analysed in 20 μl aliquots deposited directly on to the ATR FT-IR analysis stage. ATR FT-IR measurement scans were performed upon immediate deposition, with further scans carried out at set time intervals until the sample was completely dry. The set time intervals utilised for all body fluid identification analyses were; 10, 20, 30, 45 and 60 min, 2, 3, 4 and 5 h after deposition.

3. Results and discussion

Analysis of dried blood, saliva, semen and vaginal secretions with ATR FT-IR spectroscopy was conducted in order to determine the vibrational spectrum of each body fluid and the detectable components present within each body fluid type when examined in the infrared region of the electromagnetic spectrum. Three major macromolecule groups were observed amongst the body fluids; lipids (3000–2800 cm^{-1}), proteins (1700–1600 cm^{-1} and 1560–1500 cm^{-1}) and nucleic acids (1250–1000 cm^{-1}) [41]. Peaks within these regions were then assigned to specific components and vibrational modes relevant to the sample type and the observation of any additional peaks was utilised to characterise each body fluid. Tables 1 and 2 detail the components identified at each peak frequency observed within the blood, saliva, semen and vaginal secretion spectra and Fig. 1 demonstrates the typical spectra obtained for these body fluids.

3.1. Blood

The typical spectrum of blood consists of three dominant peaks in addition to some smaller peaks. The peak positioned at 3292 cm^{-1} is a broad peak which corresponds to amide A and was categorised as a medium peak. The two narrow peaks positioned at approximately 1651 cm^{-1} and 1540 cm^{-1} were categorised as strong peaks as they were the most intense peaks observed within the spectrum and correspond to amide I and amide II, respectively. The remaining peaks observed were categorised as weak peaks due their low peak intensities and correspond to; plasma lipids (2956 cm^{-1}), amino acid side chains of lipids and proteins (1456 cm^{-1}), fibrinogen/amino acid side chains (1395 cm^{-1}), amide III (1286–1320 cm^{-1}) and carbohydrates, such as glucose (1250–925 cm^{-1}). Comparisons of the spectra of the most abundant blood proteins, human serum albumin (HSA) (40 mg/ml of whole blood) and haemoglobin (Hb) (13 mg/ml) [42,59,60], with the blood spectra demonstrated that both proteins are the dominant macromolecules detected in the ATR FT-IR spectroscopic analysis of dried blood (Fig. 2).

The intra and inter-sample variation in the blood component peak frequencies was observed to be less than ± 1 cm^{-1} at all component peaks, with the exception of the amide A (± 3 cm^{-1}), methyl stretches of lipids (± 6 cm^{-1}), amide I (± 2 cm^{-1}) and amide III (± 5 cm^{-1}) peaks. The variation exhibited by these blood component peaks correlate with the expected frequency ranges [41,46,47]. The larger variation exhibited by the methyl stretches of lipids is a result of one sample exhibiting a methylene stretch, whereas the larger

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