Application note

ATR FTIR spectroscopy for the forensic tracing of blood stain age

Authors

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Introduction

Bodily fluids are often some of the most critical pieces of evidence at a crime scene, and it is vital that they are correctly identified and analyzed to obtain as much information as possible. In particular, correct identification of the type of bodily fluid present is imperative in order to properly extract DNA from the sample. These fluids are usually found as stains on a variety of substrates under different environmental conditions. Establishing the time since deposition (TSD) of a stain can help determine if a sample is relevant to the crime and verify the alibis of any individuals involved.[1-3]

Currently, the identification of bodily fluids is made with presumptive and confirmatory techniques which are non-specific and destructive. There is not one accepted technique for the estimation of TSD for bodily fluid stains.[1] For these reasons, selective, non-destructive, and fast techniques, such as attenuated total-reflection Fourier-transform infrared spectroscopy (ATR FTIR), need to be investigated and validated for their use in forensic investigation. ATR FTIR can analyze very small stains without destroying them, allowing for subsequent analysis with complementary techniques.[4]

ATR FTIR analysis of bodily fluid stains provides large amounts of data, which makes accurate data processing essential. This can be accomplished through chemometrics, or the application of mathematic and statistical methods to chemical data, as described by Weber and Lednev.[1] In this application note, home-made blood-stain reference materials were monitored with a combination of ATR FTIR techniques and chemometric methods. Orthogonal partial-least-square discriminant-analysis (OPLS-DA) models were used to differentiate the blood stains by their TSD, independently of the substrate they were deposited on.

Materials and methods

Blood from a single female donor was used to make the stains. (I.e., samples were obtained after pricking one finger with a lancet.) The blood drops were deposited on five different substrates: plywood, metal, white gauze, blue denim, and glass (Figure 1). These substrates were chosen as they are commonly encountered in many crime scenes. The samples were left to dry at ambient conditions without exposure to direct sunlight; temperature and humidity were not controlled. All the blood stains were measured after 5, 30, and 60 minutes as well as after 1, 4, 7, 14, and 28 days.

The analysis was performed with a Thermo Scientific™ Nicolet™ iS10 FTIR Spectrometer. The instrument was equipped with the Thermo Scientific™ Smart iTR™ ATR Sampling Accessory (with a diamond crystal component) and Thermo Scientific™ OMNIC™ Software v9.6 (Figure 1). A 8 cm^{-1} resolution was used with 32 scans in the 500–4,000 cm^{-1} range.

After the analysis, the resulting data was imported into The Unscrambler X v10.4 (CAMO-Aspen Technology, Houston, USA) for pre-processing; a standard normal variate (SNV) method was used to normalize the data. Then, baseline offset correction was applied prior to Savitzky-Golay smoothing (2nd order polynomial with 7 smoothing points). The matrix was imported into Microsoft Excel 2008 for easy organization. SIMCA Software v17.02 (Sartorius, Göttingen, Germany) was used to make the OPLS-DA models. OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA) was used to make the graphics.

The OPLS-DA model combines partial least squares (PLS) regression and orthogonal signal correction (OSC) methods. In OPLS-DA, there is a Y-matrix (response matrix) and a X-matrix (descriptor matrix), which is decomposed in two blocks: the correlated and the orthogonal data. OPLS-DA models can separate predictive and non-predictive variation.[5]

There are two important parameters: R^2X and Q^2. R^2X indicates how well the model explains the data, ranging from 1 (perfect fit) to 0 (the model does not fit the data). Q^2 is the predictive capacity of the model, ranging from 1 (perfect prediction) to 0 (no predictive capacity).[6]

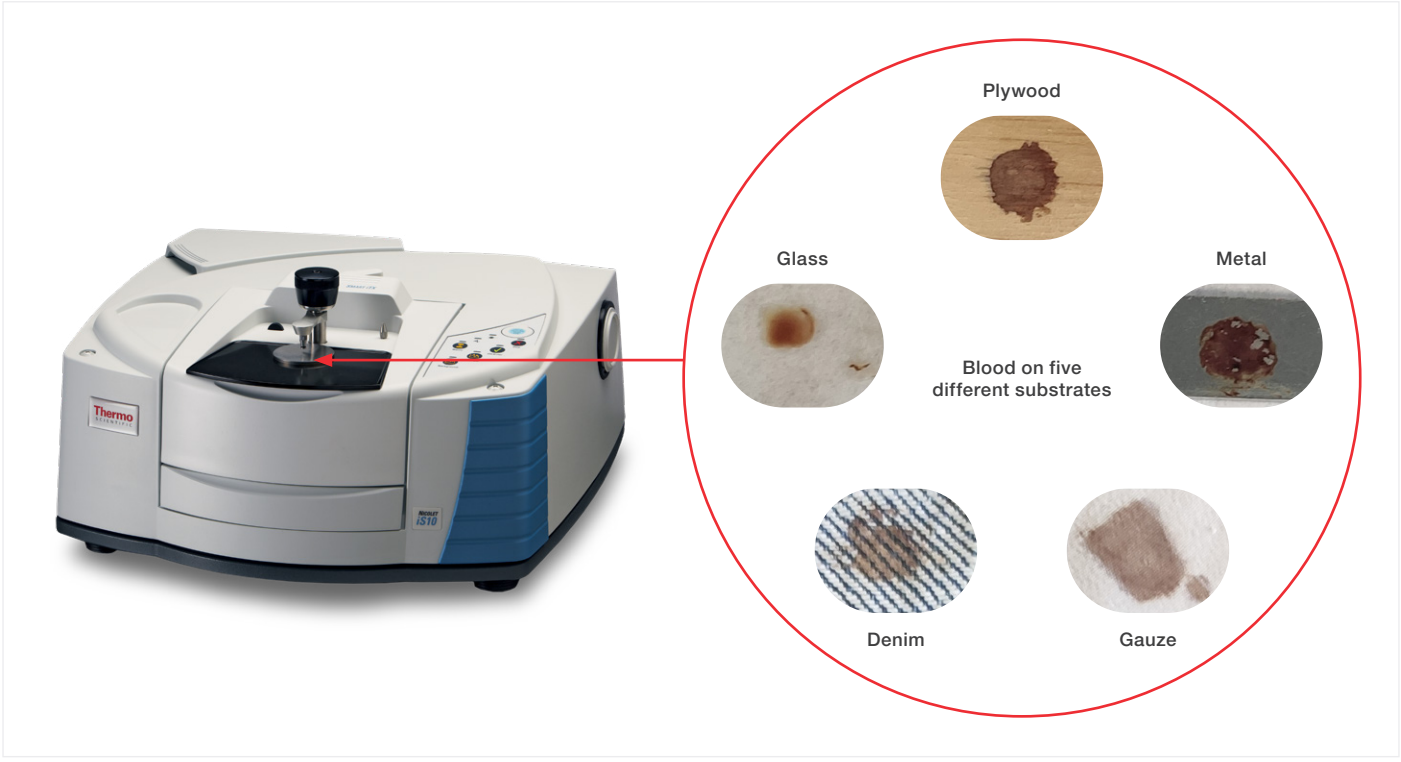

Figure 1. The Nicolet iS10 FTIR Spectrometer with the Smart iTR ATR Accessory, used to analyze blood stains made on five different substrates: plywood, metal, gauze, denim, and glass.

Results and discussion

The intensity of the blood's infrared bands depends on the nature of the substrate it is deposited on. On non-porous substrates, the fluid did not penetrate the inner layers and the substrate's characteristic bands did not appear in the spectra. Meanwhile, for porous substrates, the blood was able to penetrate all the way to the inner layers.[7] In these cases, it may not be possible to detect the blood bands with the naked eye because they are overlapped by the substrate's bands. As an example, the pure spectra of blood on non-porous glass is compared to the spectra of blood on porous denim (Figure 2). All the characteristic IR bands for blood are marked in gray.

On both substrates, blood is most identifiable in the 5 minute spectrum (marked in red). The most prominent band is water at 3,200–3,500 cm^{-1}, which is so large that it obscures many of the other bands, with the exception of the the Amide I band at 1,600–1,700 cm^{-1}. Fortunately, the samples dehydrate with time, and the rest of the bands progressively become visible as the water band decreases.

In Figure 2A, the amide bands can be seen at 698 cm^{-1} (Amide IV), 1,239 cm^{-1} (Amide III), 1,500–1,560 cm^{-1} (Amide II), 1,600–1,700 cm^{-1} (Amide I), and 3,284 cm^{-1} (Amide A). The glucose band is located at 1,082 cm^{-1} and the lipid bands are at 1,390, 2,872, and 2,958 cm^{-1}. The bands at 3,200–3,500 cm^{-1} are due to water and hydroxyl groups. In Figure 2B, the blood bands at 698 cm^{-1} (Amide IV), 1,082 cm^{-1} (glucose), 1,239 cm^{-1} (Amide III), and 1,390 cm^{-1} (lipids)[8,9] are hidden by the cellulose bands from the substrate,[10] which dominate the spectra.

Gauze and metal were the two most complex substrates to measure. In the case of gauze, the blood stain can visually be seen on the substrate, but cannot immediately be distinguished in the spectra. The OPLS-DA model could, however, correctly differentiate the pure substrate from the substrate with fluid (not shown). Due to the reflective nature of metal, spectra with a large amounts of noise were obtained for the metal substrate. Nevertheless, the characteristic bands of blood could still be seen.

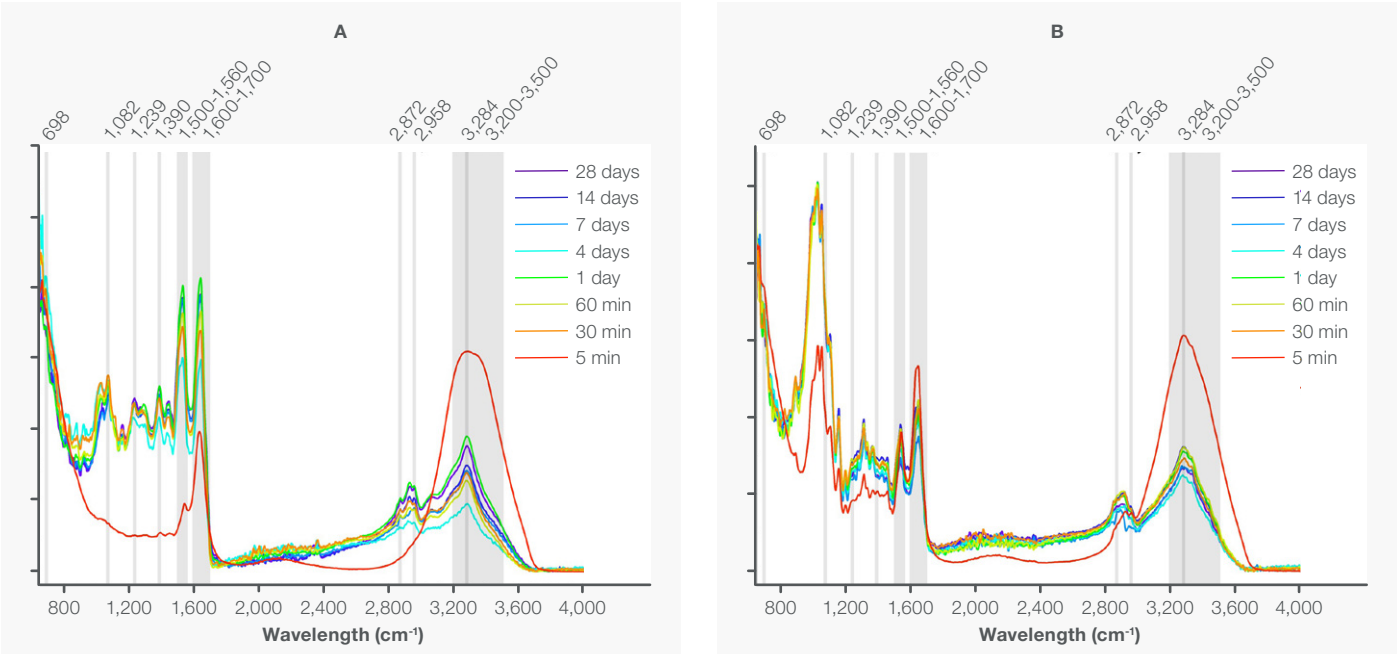

Figure 2. A) Spectra of blood stains deposited on glass, measured for 28 days. B) Spectra of blood stains deposited on denim, measured for 28 days. The characteristic bands of blood are marked in gray.

The intensity of the bands in the different spectra changes over time, but these differences are often visually indistinguishable. An OPLS-DA model, meanwhile, can clearly discriminate blood samples according to their TSD. The model (Figure 3) has an R^2X of 96.6% and a Q^2 of 59.4%. Each day can be differentiated, although the discrimination between days 4 (96 hours) and 7 (168 hours) is the least clear.

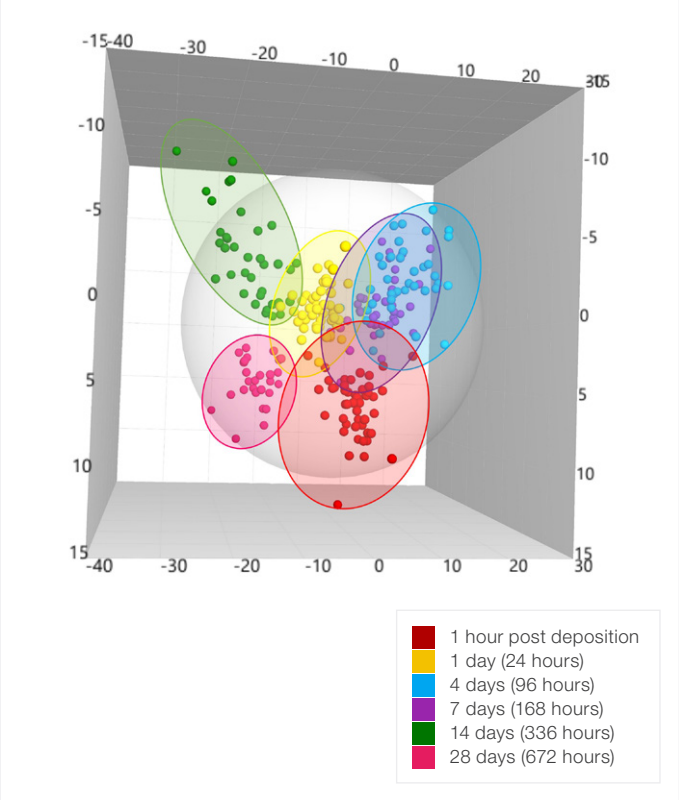

Figure 3. OPLS-DA model that discriminates blood stains by their TSD, independent of substrate. The model was created by measuring blood stains on five different substrates.

Conclusions

Combined infrared spectroscopy and chemometric modeling shows great promise as a forensic technique for the estimation of blood-stain TSD. ATR FTIR spectroscopy can be used as a blood-stain monitoring tool due to its ability to detect the characteristic bands of blood independent of the substrate they are deposited on. Additionally, these bands can be detected regardless of the time that has elapsed since the deposition of the stain.

Note: The data in this application note was collected with an older model Nicolet iS10 FTIR Spectrometer. Thermo Fisher Scientific now offers the improved Thermo Scientific Nicolet iS20 FTIR Spectrometer, with superior speed and performance over its predecessor.

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References

1. Weber, AR and Lednev, IK. Crime clock – Analytical studies for approximating time since deposition of bloodstains. *Forensic Chem* 19(3):100248 (2020)
2. Doty, KC, McLaughlin, G, and Lednev, IK. A Raman "spectroscopic clock" for bloodstain age determination: the first week after deposition. *Anal Bioanal Chem* 408(15):3993-4001 (2016)
3. Bremmer, RH, et al. Forensic quest for age determination of bloodstains. *Forensic Sci Int* 216(1-3):1-11 (2012)
4. Zapata, F, et al. Differentiation of Body Fluid Stains on Fabrics Using External Reflection Fourier Transform Infrared Spectroscopy (FT-IR) and Chemometrics. *Appl Spectrosc* 70(4):654-665 (2016)
5. Bylesjö, M, et al. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. *J Chemometrics* 20(8-10):341-351 (2006)
6. Ortiz Herrero, L. Development of new methodologies for dating in the forensic field, combining analytical techniques with multivariate regression treatments. Doctoral thesis, University of the Basque Country (2021)
7. Quinn, AA and Elkins, KM. The Differentiation of Menstrual from Venous Blood and Other Body Fluids on Various Substrates Using ATR FT-IR Spectroscopy. *J Forensic Sci* 62(1):197-204 (2017)
8. Mistek-Morabito, E and Lednev, IK. Discrimination between human and animal blood by attenuated total reflection Fourier transform-infrared spectroscopy. *Commun Chem* 3(1):1-6 (2020)
9. Mistek-Morabito, E and Lednev, IK. Discrimination of menstrual and peripheral blood traces using attenuated total reflection Fourier transform-infrared (ATR FT-IR) spectroscopy and chemometrics for forensic purposes. *Anal Bioanal Chem* 413(9):2513-2522 (2021)
10. Silva, TL, et al. Mesoporous activated carbon fibers synthesized from denim fabric waste: Efficient adsorbents for removal of textile dye from aqueous solutions. *J Cleaner Prod* 171:482-490 (2018)

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